# PAPERS

OF THE

# MICHIGAN ACADEMY OF SCIENCE ARTS AND LETTERS

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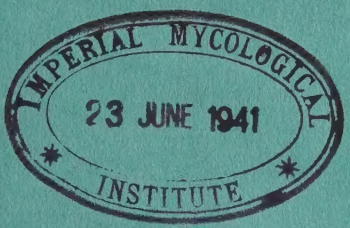
## XXVI (1940)

## PART II: ZOOLOGY



# MICHIGAN ACADEMY OF SCIENCE ARTS AND LETTERS

VOLUME XXVI (1940)

PART II: ZOOLOGY

VOLUME XXVI IS AVAILABLE IN FOUR PARTS:

PART I: BOTANY AND FORESTRY

PART II: ZOOLOGY

PART III: GEOGRAPHY AND GEOLOGY

PART IV: GENERAL SECTION

ANTHROPOLOGY, ECONOMICS
LANGUAGE AND LITERATURE

# PAPERS OF THE MICHIGAN ACADEMY OF SCIENCE ARTS AND LETTERS

EDITORS
EUGENE S. McCARTNEY
MISCHA TITIEV

VOLUME XXVI (1940)
PART II: ZOOLOGY

"Pusilla res mundus est nisi in illo
quod quaerat omnis mundus habeat."
— SENECA, *Naturales Quaestiones*

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# ZOOLOGY

# FACTORS DETERMINING TIME OF BIRTH IN THE GARTER SNAKE *THAMNOPHIS SIRTALIS SIRTALIS* (LINNAEUS) *

FRANK N. BLANCHARD AND FRIEDA COBB BLANCHARD

GARTER snakes (*Thamnophis sirtalis sirtalis*) kept in captivity primarily for the study of inheritance of melanism have proved excellent material for life-history observations. It was realized at the start (in 1922) that the genetical study would require several, or perhaps many, years and the rearing of various broods of young; accordingly, as the snakes were maintained under conditions which, if not quite natural, were at least fairly so, it was thought worth while to keep records on such topics as mating, number and size of young in relation to size and age of parent, time of birth of young, rate of growth, size and age at maturity, length of life, and so on.

When the number of snakes had increased from the original three to several dozen, a study of the factors determining the date of birth of the young was urgently needed because of the inconvenience of its varying from year to year. Usually the young were born in early August (or occasionally in late July), but some years births dragged along annoyingly through the vacation period between the summer session at the University of Michigan and the autumn opening. If the exact length of the period of development could be determined the date of birth could at least be predicted, and it might even be possible to arrange the matings at such a time that observation and care of the broods would not interfere with postsession expeditions. The subject of this paper, therefore, was studied with enthusiasm as soon as data were available.

* Contribution from the Department of Zoölogy, and Paper No. 689 from the Botanical Gardens and Department of Botany, of the University of Michigan.

The authors presented this paper at the meeting of the American Society of Ichthyologists and Herpetologists in May, 1937. The form has been slightly modified for publication, but the matter has not been changed except for the addition of the 1937 results. F. C. B.

### LIVING CONDITIONS OF THE SNAKES

The snakes with which these natural-history studies were made were kept at the Botanical Gardens of the University under conditions as natural as was practicable. They lived in a number of pits each five by six feet in area, and about four feet deep, with cement walls and natural ground bottoms. The rims of the pits are about eighteen inches above the outside ground level, and boards cover half of the top of each, for shelter. The fourteen pits adjoin one another in a single row within a grassy court a hundred feet long and enclosed by greenhouses. Here the snakes are free from disturbance. They are healthy and show no restlessness.

The temperatures in the pits are as nearly alike as they could well be under natural conditions. The pits are in a single east–west row paralleling the greenhouses, five feet from the one on the north and fourteen feet from that on the south. The two end pits are not used for the females recorded here, as each of these has a wall backed by the ground instead of by an adjoining pit, which must affect the temperature somewhat. The other twelve are as nearly identical as practicable. The temperature in them is not, of course, the same as that in natural habitats. The pits possibly provide surer winter protection, for in addition to the deep piles of leaves which they contain they are supplied with cold-frame sash. In early spring there is less sun warmth in the pits than in the woods; on the other hand, the wind also is cut off. But, though the conditions are not identical with those in nature, they are uniform throughout the pits, and the setup is the same from year to year.

The snakes doubtless forage somewhat, eating earthworms and insects which are harbored by the shelter in the pits (piles of leaves in the shady corners, bits of board and bark, old sacking, and low weeds); in the spring they may get an occasional frog, for any that become available are put into the pits; but their main food is fish. Fish obtained at market is cut into pieces of suitable size and tossed to the snakes or (if coaxing is necessary) offered on the end of a bamboo stick. During the years with which this paper is concerned (1932–37 inclusive) the snakes were well fed. Two, or sometimes three, times a week they were given all that they would eat. Generally, feeding has not been begun until after mating (at the end of April) and has ceased at about the end of September, when the snakes

become sluggish in the cool days of autumn. A regular schedule has not been followed, for feeding on a cold or rainy day was found to be a waste of effort and fish, but fairly regular feeding has been maintained. The yearly growth and good condition of the snakes indicate that they are well nourished.

The males are all kept in one pit, but in general the females are separated from one another, though each may share a pit with some immature or unmated snakes. All snakes are marked so as to be identifiable. At mating time the male is dropped gently into the female's pit and observed until copulation and separation have occurred, when he is moved to another female or returned to the males' pit.

### MATING

The dates of controlled matings have ranged from March 22 to May 13,[1] but most have fallen in the later part of April. Early matings are possible in warm spells in March and the first days of April (as observed in nature and in the pits), but to accomplish them experimentally is not easy, as one must be free to put aside other duties when the rare suitable hour arrives. Matings may take place in very early May, too; but after a certain number of good days have been allowed to pass unused the snakes seem to lose the necessary impulse, and one or both fail to respond. Many times on bright warm days in early May a male has been brought to an unmated female only to have them show complete indifference. But on bright April days, with a temperature near 70°, successive matings have easily been secured. The snakes are then intensely alert to each other, and not infrequently copulation takes place within a few minutes of the time that the male is placed in the pit with the female. Occasionally a male has copulated with two and even three females in quick succession. Matings of the same day offer a chance for comparing periods of development.

### PERIOD OF DEVELOPMENT OF THE YOUNG

In the period between copulation and birth occur: the preparation of the egg for fertilization, perhaps initiated by mating, and the migration of the egg to the oviduct; fertilization in the oviduct, prob-

[1] The snake which gave this abnormally late record had been kept in a laboratory through the winter, and apparently the seasonal rhythm of temperature was thereby disturbed for her.

ably about a month after copulation unless cold weather intervenes; and incubation during the development of the embryo. If cold weather follows copulation these events may be preceded by a dormant interval. How to recognize the divisions of this period between the beginning of preparation for fertilization of the egg and the birth of the young is not now known; consequently it is considered as a whole, without regard to when during that time fertilization takes place or how long beforehand copulation occurs, and it is here called the "period of development."

It is thought probable that the act of mating initiates the preparation of the eggs for fertilization. If the eggs were shed from the ovary and entered the oviduct for possible fertilization in the same manner whether or not mating had occurred, the exact time of mating should be immaterial, provided only that it occurred during the several weeks required for the eggs to reach the oviducts for fertilization. But it has been found that late matings produce late births. Only if preparation of the eggs for fertilization awaits the stimulus of mating (either copulation or insemination) would births be delayed, as they are, by delayed mating.

In the spring of 1934 the period of development was computed from the data at hand, and a confirmation in August was awaited. But, surprisingly, *all* the broods appeared *at once* during the last week of July, instead of during two weeks in mid-August as predicted! It was altogether the earliest season so far recorded; furthermore, it was hot, steadily hot. A connection suggested itself. Now, with the additional data of the contrasting, equally unusual, cool season of 1935, the birth records have been summarized and correlated with the temperature records at the Astronomical Observatory of the University of Michigan.

Twenty-seven normal broods born during four consecutive summers (1932–35 inclusive) are recorded here (Table I). Offspring of earlier years are not included because the mating dates are not known or because young were born under differing conditions, when the females had been moved to cages because of lack of accommodations. This shift resulted in abnormal broods, often dead at birth, and born over a period of days or weeks.

The history of the preceding season is known for all females used, with the exception of three in the series of 1934. In fifteen instances the females had mated and had had young the previous summer; in

## TABLE I

DATES OF MATING AND OF BIRTH OF RESULTANT BROODS OF *THAMNOPHIS SIRTALIS SIRTALIS* IN THE YEARS 1932–35 INCLUSIVE

| 1932 | | | | 1933 | | | | 1934 | | | | 1935 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Snake | Date of mating | Date of birth | No. of days between mating and birth | Snake | Date of mating | Date of birth | No. of days between mating and birth | Snake | Date of mating | Date of birth | No. of days between mating and birth | Snake | Date of mating | Date of birth | No. of days between mating and birth |
| A | Apr. 19 | July 29 | 101 | A | Apr. 4 | July 30 | 117 | V | Apr. 9 | July 24 | 106 | V | Mar. 22 | Aug. 20 | 151 |
| Q | Apr. 19 | Aug. 4 | 107 | W | Apr. 10 | July 31 | 112 | 3* | Apr. 9 | July 24 | 106 | B | Mar. 22 | Aug. 22 | 153 |
| N | Apr. 19 | Aug. 11 | 114 | V | Apr. 10 | July 29 | 110 | W | Apr. 9 | July 25 | 107 | AG | Apr. 26 | Aug. 12 | 108 |
| R | Apr. 20 | Aug. 5 | 107 | B | Apr. 10 | Aug. 2 | 114 | B | Apr. 9 | July 26 | 108 | 1 | Apr. 26 | Aug. 20 | 116 |
| B | Apr. 20 | Aug. 10 | 112 | R | Apr. 21 | July 30 | 100 | Q | May 1 | July 24 | 84 | 3 | Apr. 26 | Aug. 25 | 121 |
| W | Apr. 22 | Aug. 6 | 106 | P | Apr. 24 | July 30 | 97 | 1* | May 1 | July 26 | 86 | | | | |
| V | May 13 | Aug. 24 | 103 | AD | Apr. 29 | July 29 | 91 | AC | May 1 | July 29 | 89 | | | | |
| | | | | | | | | 2* | May 1 | July 30 | 90 | | | | |

* This is one of three females whose history of the previous summer is not known. In all other instances the female was under control for the full preceding season, and there had been no chance for mating since the preceding spring.

three they had mated but had failed to bear; four females had neither mated nor borne in the season preceding the experiment; in two instances it is known only that the snakes had had no offspring. Most of these females appear in the records of two, three, or all four years of the experiment.

### RECORD OF EACH YEAR, 1932–35 INCLUSIVE

#### *1932*

In 1932 six matings were made within three days (April 19–22). These gave broods over a period of fourteen days (July 29—August 11). The three matings of the earliest day (April 19) gave births at the two extremes of the fourteen-day period (one on July 29, one on August 11) and one birth between. It may therefore be concluded that this amount of variation in period of development (fourteen days) is due not to differences in time of mating but to the individuality of the snakes or to some other uncontrolled factor. Certain constantly "early" snakes had been noted before and used as indicators, giving warning as to when the bulk of the broods were due.

In this year there was also an unusually late mating, on May 13.[2] The resulting young were born late, on August 24; still, the number of days between copulation and birth (103) was within the range of variation (101–114 days) of the six females which mated at the usual time. This fact suggests that for this snake in this summer 103 days were necessary from copulation to birth, and that this brood was delayed by late mating. But as the birth date was only nineteen days later than the "average" date for the group of six, it may be estimated that this group had only nineteen days developmental start over the May 13 young; that is, their development started about nineteen days before May 13, or about April 24.

The data for this season are given in Table II.

#### *1933*

The next year, 1933, the mating dates were on the whole somewhat earlier, ranging from April 4–29 instead of from April 19–22 as in 1932. The births, too, were earlier (July 29—August 2), giving the same average period between mating and birth of young — 107 days in 1932, 106 days in 1933. This consistency in average

[2] See footnote 1, page 163.

TABLE II

PERIOD OF DEVELOPMENT AND TIME OF BIRTH OF *THAMNOPHIS SIRTALIS SIRTALIS* IN RELATION TO TEMPERATURE IN THE YEARS 1932–35 INCLUSIVE

| Year | 1932 | 1933 | 1934 | 1935 |
|---|---|---|---|---|
| No. of instances | 6 | 7 | 8 | 5 |
| Dates of mating | Apr. 19–22 | Apr. 4–29 | Apr. 9 and May 1 | Mar. 22 and Apr. 26 |
| Dates of birth | July 29–Aug. 11 | July 29–Aug. 2 | July 24–26 and 24–30 | Aug. 20, 22 and 12–25 |
| Average date of birth | Aug. 5 | July 30 | July 25 and 27 | Aug. 21 and 19 |
| Computed date of start of development | Apr. 24 | Apr. 29 or later | Apr. 29 | Apr. 26 or later |
| No. of days of development (extremes) from computed date of start | 96–109 | 91–95 | 84–90 and 86–88 | 108–121 |
| No. of days variation due to uncontrolled factors | 14 | 5 | 6 | 14 |
| Average no. of days of development from computed date of start | 104 | 93 | 87 | 116 |
| Average temperature for 3-month period (May 1—Aug. 1) | 67° | 69.5° | 71.7° | 64.5° |
| No. of degrees hotter than 27-year average (65.7°) | 1.3° | 3.8° | 6° | — 1.2° |
| No. of degrees hotter than in coolest of the four seasons (1935) | 2.5° | 5° | 7.2° | .. |
| No. of days shortening of period of development as compared with 1935 | 12 | 23 | 29 | .. |
| No. of days shortening of period of development per degree increase in average temperature | 4.8 | 4.6 | 4 | .. |
| | Average 4.5 | | | |

time between mating and birth, in spite of the difference in mating dates, suggests a fixed interval as the period of development. *But the earliest mating, April 4, and the latest, April 29, gave broods at the same time — July 30 and 29 respectively.* Apparently there is no advantage from the earlier mating. The fact that all births came within a five-day period (July 29—August 2) would indicate that in all females, despite differences in date of mating, the period of development started at about the same time. If this is so, and since the matings ranged from April 4 to 29, the conclusion is that development did not start before April 29.

In 1933, as in 1932, three matings of the same day gave the extremes in the season's birth dates (July 29 and August 2) and a date between (July 31), thus indicating some individual differences in the snakes or their pits.

So in 1933, instead of a period of development of 106 days (average time between copulation and birth) there is, rather, a dormant period, until April 29 at least, and then a developmental period of 93 days or less. And the consistency between 1932 and 1933 in the average period between mating and birth (107 days in 1932, 106 in 1933) is mere coincidence (depending on the date on which we happened to cause the matings), the actual periods of development being 104 and 93 days, respectively.

The data for this season are given in Table II.

*1934*

In the season of 1934 the eight matings were made on two dates three weeks apart (April 9 and May 1). This gave an excellent chance for comparison. *All* the births came in *July* (July 24–30)! The matings of April 9 gave young on July 24, 25, and 26; those of May 1, on July 24, 26, 29, and 30. That is, those of April 9 averaged only two days earlier (July 25) than those of May 1 (July 27), the earlier group apparently having but two days' start in spite of three weeks' advantage. It might be figured, then, that in 1934 development started two days before May 1, on April 29. This assumes that the two days in April are equivalent to the two days in July, which of course they may not be.

If, as figured, April 29 is set as the starting point of development, then the period was 87 days — April 29 to July 25 for the earlier group, and May 1 to July 27 for the second group. One could not

fail to be aware that this unusually short period was coincident with an exceedingly hot summer. No season could have been better suited to the needs of the experiment, and he would have been dull indeed who failed to take the hint.

The data for this season are given in Table II.

*1935*

In the next spring, 1935, a warm spell in March gave an opportunity for two extremely early matings (March 22). The other three matings of the season were exactly five weeks later (April 26). The broods from the early matings were born on August 20 and 22 (average, August 21); those of the matings of five weeks later, on August 12, 20, and 25 (average, August 19). Thus the early matings gave no advantage — in fact their births averaged two days later, a variation attributable to the individuality of the snakes or to minor differences in environment. In this season, then, we may be justified in setting April 26 (the later mating date) as the earliest date at which development could have begun. And if it began at this date, the period of development averaged 116 days — an unusually long time.

In this season, 1935, a wild garter snake was captured in copulation on March 16. Her brood was born 171 days later, on September 3. Since this was her first season of captivity in the pits it may be that the birth of her brood was delayed by conditions to which she was unaccustomed; but as the period of captivity before birth of young was almost half a year she was presumably adapted to her surroundings long before the brood was born. This is the most extended period we have observed, for birth was 130 days after the date determined for beginning of development.

The data for this season are given in Table II.

## CONCLUSIONS FROM OBSERVATIONS DURING THE FOUR SEASONS 1932–35 INCLUSIVE

Very early matings (March and early April) have not resulted in earlier broods. Late matings (in May) have postponed birth by about the number of days that the matings were delayed past the last week of April, the time when the snakes ordinarily become continuously active. These facts are brought out in Figure 1 (p. 170).

The mating of May 13 in 1932 gave a birth delayed nineteen days,

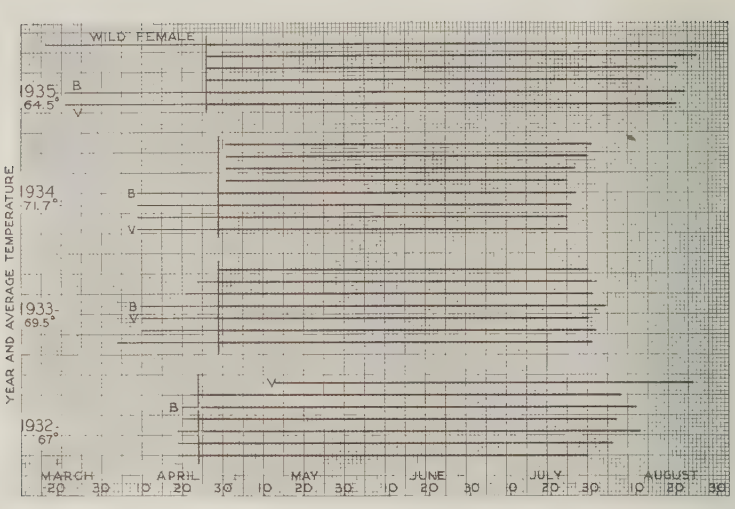


FIG. 1. Diagram showing dates of mating, birth of young, and periods of development of *Thamnophis sirtalis sirtalis* in the years 1932–35 inclusive, with average temperatures for the three-month period May, June, and July. Each horizontal line represents the record of a brood, starting at the date of copulation and ending at the date of birth. The vertical lines indicate for each year the estimated beginning of the period of development. The records of females B and V, the only snakes for which we have data for all four years, are indicated by letters. It is evident that (1) the warmer the summer the shorter the period of development, (2) very early mating does not bring about correspondingly early birth, (3) mating later than at the usual time postpones the date of birth

and those of May 1 in 1934 a handicap of two days, bringing the starting dates definitely to April 24 and 29 respectively. But the corresponding dates in 1933 and 1935 cannot be stated so certainly. We can only guess that the dates given as those *earlier* than which development did *not* start in 1933 and 1935 (April 29 and 26) are probably fairly close to the date at which it did start. Evidently under conditions of the experiment the last week in April may be considered the critical time.

But the records show that matings of late April, the common time, do not in different years produce young at the same date, and that though the periods of development may regularly begin in late April there is great variation in birth dates. In an extremely early

season (1934) the young may be born a month earlier than in an extremely late season (1935), the period of development in the early season being only three quarters that in the late season. In the four consecutive seasons recorded the days required for development have been figured as 104, 93, 87, and 116 respectively (see Table II).

### TIME OF BEGINNING OF DEVELOPMENT

A weakness of these computations is that the conclusions depend directly on the estimated date of the starting of preparation for fertilization, and this has not been accurately determined. It has been assumed, for lack of information and because of working with averages, that a day at the end of the period, in August, is equivalent to one at the beginning, in April or early May, but this doubtless is not true. The error, however, must be trifling.

Also, it has been assumed that in spring there is no development until a certain day, when it suddenly starts at full speed. It may be that there is, instead, a period of slow and gradually accelerating internal activity, and that the date of actual beginning is unimportant. Yet with our usually rather sudden seasonal change about May 1, when the snakes lose their torpor and become lively again, it seems probable that there is a sudden burst of activity within them comparable to the sudden bursting of the buds on late-leafing trees which during April have shown practically no change. And the fact that in 1934 the April 9 group had an actual developmental start of only two days over that of the May 1 group shows that if activity began right after mating it was so slow as to be practically negligible or it was faster and confined to a very few days of the month. No evidence is now seen to indicate how the figures should be modified in this respect.

### RECORDED TEMPERATURES OF THE FOUR SEASONS 1932–35 INCLUSIVE

The average temperatures (Fahrenheit) for the period May 1 through July 31 (practically the period of development) are as follows for the years 1932–35: 67°, 69.5°, 71.7°, 64.5°.

These figures were derived from the monthly averages recorded at the University Observatory, where the mean of the daily extremes was taken as the average for the twenty-four hours, and the average of the daily means was taken as the monthly average.

Not only was the average of the 1934 season high for the three-month period, but each separate monthly average was a highest record for the twenty-seven years recorded at the Observatory. The season was an extreme variant, being 6° hotter than the average for the twenty-seven-year period and 7.2° hotter than the coolest of our four years, 1935. Perhaps in a century one would not be favored again with two consecutive years so contrasting.

### RELATION OF TEMPERATURE AND LENGTH OF PERIOD OF DEVELOPMENT

The difference of 7.2° in average temperature between 1934 and 1935 is coincident with a twenty-nine-day difference in time required for development, or four days per degree of temperature. Comparing the other two seasons, 1932 and 1933, with the cool season of 1935, we find a shortening of twelve days for 2.5° in 1932 (or 4.8 days per degree of temperature), and twenty-three days for 5° in 1933 (or 4.6 days per degree). From these four years it is concluded, then, that a variation of one degree in temperature causes a difference of four and a half days in the time of birth.

### OTHER FACTORS WHICH MAY AFFECT TIME OF BIRTH

The temperatures used are those of the Observatory, nearly two miles from the Botanical Gardens. No records were made of temperatures in the pits, for it was only after a glaring correlation appeared between very early birth and such unusual summer heat that a thermometer was unnecessary to detect it, that the interest in temperatures was aroused.

But even had air and soil temperature readings been made within the pits, nothing would be known about the amount of heat absorbed by the snakes, for they were, of course, always free to lie basking in the sun or to seek cool spots under boards in the shade. As a matter of fact, it is quite possible that snakes absorb more heat when temperatures are not at their highest, for when it is cool or merely warm they seem to enjoy the sunshine, but during the hot weather they avoid it during most of the day.

Whether the average air temperature in the pits is above or below that at the Observatory is not now known. It is assumed, however, that with variation in the Observatory temperatures there is a corresponding change in the pit temperatures. Possibly on sunny

days the relation between pit and Observatory temperatures is not the same as on cloudy days. It may be that there was great variation in the amount of sunshine in the four years under consideration and that sunshine is an important factor. Perhaps maximum daily temperatures as well as average daily temperatures should be considered. In other words, it is fully realized that because of ignorance concerning them, many pertinent factors have been left out of this consideration. There is, nevertheless, a close correlation between the temperatures recorded at the Observatory and the date of birth of young in the pits.

### VARIATIONS IN BIRTH DATES DUE TO THE INDIVIDUALITY OF THE SNAKES OR TO SLIGHT DIFFERENCES IN THEIR SURROUNDINGS

The variations in birth dates attributed to the individuality of the female snakes (or to minor differences among their pits) are as follows:

| *Year* | *Average developmental period* | *Variation* |
|---|---|---|
| 1932 | 104 days | 14 days |
| 1933 | 93 days | 5 days |
| 1934 | 87 days | 6 days |
| 1935 | 116 days | 14 days |

As would be expected, the shorter the period of development, the more concentrated the dates of births; or, the longer the development, the more scattered the births.

These variations may be due to several factors. Environments are not identical. Perhaps a slightly higher leaf pile in one pit is struck by the sunshine earlier in the morning, allowing the snake a longer basking time. Perhaps feeding is involved. Possibly the size of the brood influences the time of birth. At any rate, the records of three females, B, V, and W, each of whom remained in her own pit through three seasons, show that the snakes or the pits do have constant individuality in respect to the length of the period of development.

For three years in succession the females B, V, and W happened to be mated all on the same day; they thus afforded a chance for recording individuality. In 1933 all three mated on April 10; in 1934, on April 9; and in 1935, on March 22. This is the only such series obtained, but the results are rather convincing (see Fig. 2, p. 174). In 1933, a moderate year, V had young July 29, W two days

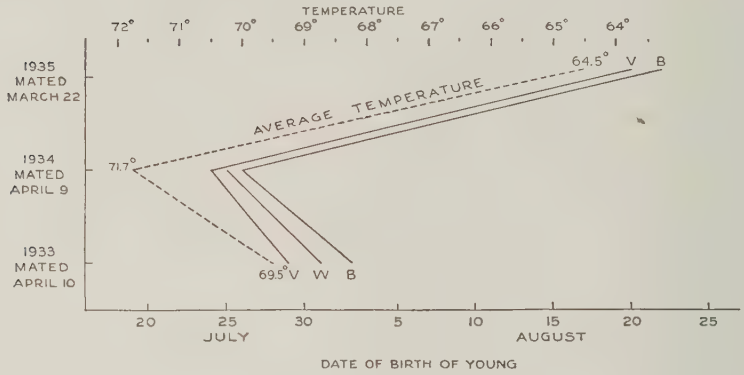


FIG. 2. Diagram showing consistency of three females of *Thamnophis sirtalis sirtalis*, V, W, and B, in regard to comparative length of periods of development of their young. In each of the three years the three females mated on the same day

later, and B two days later still. In 1934, the hot year, V had young July 24, W one day later, and B one day later than that. In 1935, the cool year, V had her brood August 20, and B two days later. The three maintained their proper sequence (except that W unfortunately died before the last record was made) and thus demonstrate a minor variation in length of period of development which is due to details of housing or, perhaps more probably, to inherent differences in the constitution or behavior of the snakes.

### CONFIRMATION IN 1937 OF COMPUTED RELATIONSHIP BETWEEN TEMPERATURE AND PERIOD OF DEVELOPMENT

In 1936 the data were so scant and imperfect as to be valueless.

In 1937 three matings were successfully carried through. Young from matings of April 29, May 3, and May 3 were born August 19, 17, and 16, respectively — 112, 106, and 105 days after mating. But the first mating gave the latest brood, which indicates that development did not begin, at earliest, until the time of the later matings, i.e. May 3. Yet this is a late starting date, if we judge from the evidence of other years; consequently it seems reasonable to assume that starting was no later than this and to set May 3 as the date from which to reckon. This gives 108, 106, and 105 days, with 106 days as the average length of the period of development in 1937.

This season, instead of looking up the weather records first and then determining as before the variation in number of days with the variation in average temperature, we reversed the process, and, calculating from the periods and the scale of variation already determined from the previous years (four and a half days variation with each degree of temperature), we made a reckoning as to what average temperature should produce this result (a 106-day period). From Table II it was computed that with a period of 106 days the average temperature for the three months must have been a fraction under 67°. Compared with 1932 (temperature 67°), the period in 1937 was two days longer, that is (allowing four and one-half days for one degree) four ninths of a degree lower in temperature, or approximately 66.6°. Compared with 1935 (temperature 64.5°), it was ten days shorter, that is, about two and two ninths degrees warmer or approximately 66.7°. (These are the two years most similar to 1937.)

After thus predicting that the average for the period May, June, and July in 1937 would be found to be about 66.6° or 66.7°, we consulted the Observatory records. The average given there is 66.3°.

## SUMMARY

Garter snakes living under comparatively uniform and natural conditions (in pits with thirty square feet of ground surface) have provided data on many aspects of life history, as well as on the genetical problems for which they were originally kept. The conditions in the different pits housing the snakes discussed in this paper were very similar, and constant from year to year.

In these surroundings late April is the natural time for mating, but effective copulations have occurred in a warm spell in March. After a certain number of appropriate days have passed, the snakes, though still unmated, seem to lose the necessary impulse. Sometimes a mating has been secured in early May, but only with great patience, and by that time working with the snakes is generally fruitless.

By "period of development" of the young is meant here the time between the start in the egg of preparation for fertilization (which happens probably a month after mating) and the time of birth of the young, without regard to either the point at which fertilization occurs or how long beforehand copulation takes place. It is supposed that mating may initiate the period of development.

We have found that very early matings are not followed by cor-

respondingly early births — that there is no time gained by mating before late April, when the continuously warm weather sets in. But births from matings after the beginning of the last week of April (approximately) are delayed accordingly. It is concluded that under the conditions of the experiment the period of development starts in late April and that matings in earlier warm spells are followed by developments so slow as to be negligible or by a pause until the weather warms up.

The periods of development of twenty-seven normal broods born during four consecutive summers (those of 1932–35 inclusive) are computed here, and the average for each season is compared with the average temperature of the period (May, June, and July) as recorded at the University Observatory.

In the different years the computed period of development has varied greatly — from 87 days in 1934 (an extremely hot summer) to 116 days in 1935 (an unusually cool season). The twenty-nine–day difference in the two seasons is coincident with a difference of 7.2° in the average temperatures during the periods of development, a difference of four days per degree.

From comparison of the four years under discussion, it is estimated that an increase of one degree in the average temperature brings the birth date four and a half days earlier.

University of Michigan

# THE INHERITANCE OF MELANISM IN THE GARTER SNAKE *THAMNOPHIS SIRTALIS SIRTALIS* (LINNAEUS), AND SOME EVIDENCE OF EFFECTIVE AUTUMN MATING*

FRANK N. BLANCHARD AND FRIEDA COBB BLANCHARD

COAL-BLACK specimens of the garter snake *Thamnophis sirtalis sirtalis* (Linnaeus) have been collected at several localities on the shores of Lake Erie. E. B. S. Logier, of the Royal Ontario Museum of Zoology, Toronto, has reported a garter-snake population at Long Point, Norfolk County, Ontario, of whose members he judges that about one third are black (1931). Previously (1925) he recorded specimens from Point Pelee, Ontario, as did also Clyde Patch (1919). Roger Conant (1938) has had black specimens from various places in the marshes which rim the southwestern shore of Lake Erie, about a score having come to his attention; he writes that Point Place (part of Toledo on the lake shore) and Little Cedar Point are good places to find them, and that several have been taken in Toledo city parks. It would seem reasonable to suggest that further collecting may show a continuous range of the melanistic phase of this snake on the shores of Lake Erie.

## APPEARANCE OF THE MELANISTIC GARTER SNAKES

These snakes may be described as completely black except for a pure white throat. From above they look velvety black, the head

* Contribution from the Department of Zoölogy, and paper No. 688 from the Botanical Gardens and Department of Botany, of the University of Michigan.

Except for a few findings of the seasons of 1938 and 1939, the facts given in this paper were known to F. N. Blanchard and the interpretations accepted by him. Beginning in 1922 the study recorded here was shared by us for fifteen years, but I have been responsible for the working of our data into a finished manuscript. Though the writing of this paper, concerned mainly with genetics, would have fallen naturally to my lot it suffers, needless to say, for lack of his suggestions and criticisms. F. C. B.

appearing iridescent. The under side of the head and throat (parts which even though they are of the lightest shade still have some color in normal specimens) are pure white, and the white is continued on several of the foremost belly scutes. Then there is an abrupt change to bluish black ventrals with lighter splotches, the light areas decreasing in size and intensity posteriorly until they disappear entirely; from that point the belly is blue-black.

The sides of the head and neck are the only parts, in the dozen specimens on which this description is based, to show a trace of the normal color pattern. The upper labials are brownish gray with black flecks; the white lower labials have a very fine dark stippling. The sides of the neck adjacent to the pure white belly scutes (the region normally of most intense color, often bright yellow or orange) are tan-gray with black flecks. This little area of brown-gray, the only sign of any color in the black snakes which we have seen, is variable in amount, sometimes hardly noticeable and sometimes extensive enough to show a trace of pattern, an indistinct stripe on the neck. Our dozen specimens in hand, and the more than a hundred young which they have borne, vary not in the quality of the black but only in the extent of the pure white at the throat and of the small amount of tan-gray on the neck, which might be expected to be sometimes extensive enough to show a normal pattern in the neck region.

When the skin is loosening for sloughing and the eyes are milky, the snakes lose their sleek glossy blackness, becoming a decidedly brownish rusty black, and the ghost of a dorsal stripe is somehow visible. At this time one would hesitate to call the snakes black. After shedding they are again velvety black.

That the distinction between black and normal pattern may be lost with preservation has been mentioned by Logier (1931). This change is comparable to that before shedding.

These melanistic snakes are black from the beginning, just as black at birth as they are as adults. In many species of snakes dark individuals grade from normal, and some darken with age, losing the bright colors and markings of their early life. Eventually they may become very dark and may be loosely called "black"; but this deepening of color is not true melanism, and the result is very different from the velvety, almost blue-black of melanistic snakes.

The intense blackness of these garter snakes is not modified in

breeding, for in broods containing both normal and black young the black are at birth just like the black ancestor, and the striped ones are perfectly normal.

AN UNSUCCESSFUL ATTEMPT TO PRODUCE A HYBRID $F_2$ GENERATION

The experimental hybridizing of black and normal garter snakes started with a black male caught near Ann Arbor in June, 1922 (believed to be the only black specimen known from the vicinity), and two normal females, A and B, taken in the same region in the autumn of that year. The three snakes spent the winter and spring in an enclosure together, and in August each female bore a brood of normal, striped young. These two broods were tended carefully.

In the following three years the same two females bore five broods of young, all striped, offspring of the black male; but, unfortunately, we depended on the two broods of the first year alone to produce an $F_2$ generation, and the later young were not tended so carefully, with the result that none reached maturity.

As all the $F_1$ broods had been entirely normal and striped, there was an expectation of segregation of striped and black in the next generation, with striped predominating — presumably in a ratio of 3 : 1. In 1927 an $F_2$ generation of twelve young appeared, surprising in that it contained no black individuals. This result of course led to misgiving as to the parentage of the $F_1$ snakes, suspicion that the black snake was not father of the 1923 broods. In 1928 there were two more $F_2$ broods, from females descended from B with males descended from A, one of eight young, one of nine — all striped. In 1932 another $F_2$ brood (this time from parents which were both from A) still failed to give any black ones. Table I (p. 180) records the crosses of the 1923 offspring of females A and B which, on the assumption that black is a recessive character, were expected to produce some black young.

It happened that those which reached maturity of the 1923 brood from A were males (except female C) and that those from B were females, so that most of the $F_2$ broods were necessarily descended from both A and B. One, however, that of 1932, was from A alone.

The fact that the 1932 brood, both of whose parents were offspring of A, was entirely normal shows that melanism, if a simple recessive, was not carried by A's 1923 brood. There was no $F_2$

## TABLE I

RECORD OF THOSE PROGENIES FROM THE 1923 OFFSPRING OF FEMALE GARTER SNAKES A AND B (SUPPOSEDLY $F_2$ GENERATION FROM THE BLACK MALE) WHICH WERE EXPECTED TO SHOW A REAPPEARANCE OF THE BLACK PHASE (IN THE PROPORTION OF 3 STRIPED TO 1 BLACK IN THE FOUR $F_2$ GENERATIONS AND 1 STRIPED TO 1 BLACK IN THE THREE BACKCROSSES)

The absence of black individuals in the second generation indicates that the original black male confined with these females from the autumn of 1922 was not the father of their broods born in 1923, as had been supposed.

| Year | Generation of brood | Female* | Male* | Young (all striped) | Unfulfilled expectation of ratio of striped to black |
|---|---|---|---|---|---|
| 1927 | $F_2$ | $F_1$ L from B† or $F_1$ C from A | $F_1$ D or E from A† | 12 | 3 : 1 |
| 1928 | $F_2$ | $F_1$ H from B | $F_1$ E from A | 8 | 3 : 1 |
| 1928 | $F_2$ | $F_1$ M from B | $F_1$ D from A | 9 | 3 : 1 |
| 1932 | $F_2$ | $F_1$ C from A | Sib D | 13‡ | 3 : 1 |
| 1932 | Backcross to black ♂ | $F_1$ H from B | Black TB | 8‡ | 1 : 1 |
| 1932<br>1934 | Backcross to black ♀ | V<br>V | $F_1$ D from A<br>$F_1$ D from A | 12<br>51 | 1 : 1 |

* The snakes referred to and their origins are as follows:

| *Collected* | *Raised in captivity* |
|---|---|
| Original black ♂, caught near Ann Arbor in June, 1922 | C, D, E } from A, 1923 |
| TB, new black ♂, caught near Toledo in 1930 | |
| A ♀ (striped), caught near Ann Arbor in autumn, 1922 | |
| B ♀ (striped), caught near Ann Arbor in autumn, 1922 | H, L, M } from B, 1923 |
| V ♀ (black), caught near Toledo and captive since 1930 | |

† Not realizing that these little snakes had reached maturity, we had kept an $F_1$ group together during the summer; accordingly, when this brood was born, we could only say that it was an $F_2$ from the original females. Though the exact parentage was not known a record of the appearance of the females led to the conclusion that the mother was L (from B), rather than C (from A); the father, whichever individual he was, was of the 1923 brood from A. So presumably this brood was descended from both A and B.

‡ This brood was born under unnatural conditions, during a period of six weeks instead of all on a single day. The young died at birth or before, but there was no trouble in determining the color.

brood from B alone. If melanism was carried by B's 1923 brood, though not by A's, the $F_2$ generation descended from both A and B could not contain black, for A's offspring were pure dominants. This leaves the possibility that B's offspring did carry melanism. A backcross of her offspring to a black snake throws some light on this. Unfortunately the original black male had died, and the conclusions must rest on the assumption that the new black male (TB, from near Toledo) had the same black factor as did the original one used in the experiment, which there is no reason to doubt, nor yet perhaps any reason to believe. If this factor was the same the young from a black snake mated with B's 1923 offspring should show whether the latter carried melanism — if they did the progeny should be of striped and black in equal numbers, if not they should all be striped. The backcross of this nature shown in Table I ($F_1$ ♀ H (from B) × black ♂ TB) produced only normal young, indicating that B's 1923 brood did not carry melanism.

Thus it appears that there was no black in the constitutions of the 1923 broods of either A or B (or at least in those members of the broods which became parents), and that the black male was not the parent of the broods though he alone was confined with the females since the autumn of 1922. Evidently both females had mated before they were caught in the autumn, and the autumn insemination was effectual for the following summer's broods.

### AUTUMN MATING

Evidence of effective autumn mating has been obtained several times. A group of six females collected on October 29, 1933, was deprived of the chance for spring mating in 1934. One of these females bore a brood of twenty-two young on August 1, 1934; and on August 7 a brood of seventeen young was borne by another. In 1936 several females were caught very late in the autumn, as near as possible to the time of hibernating. Of this group six lived successfully through the winter and following summer. Of the six one had a brood of thirteen young on August 19; another dropped fourteen large hard yolks on September 10; the others gave no young, and dissection in late autumn showed neither embryos nor yolks. Again, in the summer, 1938, a female collected the previous autumn gave a brood with no mating after capture.

It is well known that these snakes often show mating behavior

in the autumn, and actual copulation has been seen. In the fall of 1936 a spontaneous copulation was observed in a pit in which a number of males and females had been together for several weeks. Two normal matings were secured on September 29, 1933, by introducing a male into the female's pit, as is done for the spring matings. One of the two females died during the winter. The other, which had been in copulation for only nine minutes — a doubtfully sufficient period — gave no brood the following summer, but neither did she from a normal spring mating the next season.

It is supposed that insemination is the stimulus to growth of the eggs, which several weeks later enter the oviduct and are there fertilized. If autumn mating results in a progeny, either it must be early enough for the eggs to have several weeks in which to reach the ovary, where, fertilized, they remain dormant during winter; or, as is perhaps more likely, the sperm must live through the hibernation period so that fertilization occurs in the spring.

That sperm can remain functional in the female from autumn to spring is easy to believe. Young can be produced by a copulation made during the very early spring in an abnormally warm spell which is followed by the normal cold weather; this shows that sperm can survive in the female during at least a short hibernation. For example, on March 22, 1935, the temperature [1] reached 60°, and mating occurred. After this warm spell there was a period of cold weather, and the temperature did not again reach 60° until April 19, when 64° was recorded. The broods from the matings of March 22 were born no earlier than those from matings of five weeks later (April 26), a fact which indicates that the sperm were merely harbored in the female during a brief period of hibernation. Such an occurrence makes it easy to imagine autumn mating being effectual.

Thus, though the autumn mating of the females A and B in 1922 set us back many years in the genetical problem of the inheritance of melanism and retarded us in getting a segregating $F_2$ generation, something equally valuable was thereby learned. It will be interesting to know how frequently mating occurs in the autumn, how likely it is to be effectual, and whether it prohibits a spring mating or if two copulations may precede one brood.

[1] Daily maximum temperature as recorded at the Astronomical Observatory of the University of Michigan.

TABLE II

RECORD OF BLACK FEMALE GARTER SNAKE V

| Year | Date of mating | Duration of contact (in min.) | Mate | Date of birth of young | Young | | | Length in mm. in Sept. |
|---|---|---|---|---|---|---|---|---|
| | | | | | Total | Alive | Color | |
| 1932 | May 13* | .. | D (normal) † | Aug. 24 | 12 | 11 | normal | 760 |
| 1933 | Apr. 10 | .. | TC (black) | July 29 | 23 | 22 | black | 830 |
| 1934 | Apr. 9 | 24 | D (normal) | July 24 | 51 | 51 | normal | ... |
| 1935 | Mar. 22 | 15 | Brown (abnormal in color but not black) | Aug. 20 | 43 | 43 | normal | 848 |
| 1936 | May 1 | 18 | Brown (abnormal in color but not black) | No brood | | | | 886 |
| 1937 | May 3 | .. | 2–1 (appearing normal but carrying black factor) ‡ | Aug. 17 | 48 | 48 | 26 normal, 22 black | ... |

* The snake was kept, in a cage, in room temperature throughout the winter of 1931–32, and the abnormally late mating in 1932 may have been made possible by her abnormal winter experience. In no other instance have we secured mating so late in May.

† See Table I. This male was a member of A's 1923 brood, which was expected to carry melanism but was shown by breeding to be normal.

‡ This normally striped male was born in 1931 in the brood of ♀ A (normal) × ♂ TB (black).

### INHERITANCE OF MELANISM

When, in the summer of 1927, suspicion first arose that the 1923 broods were not the offspring of the black male it was too late to repeat the work, for the black male died that autumn (1927). But in 1931 we were enabled to start again, with black snakes from the vicinity of Toledo, kindly supplied by Mr. Conant.

The second attempt to learn the manner of inheritance of melanism was made with three black snakes: two males, designated TB and TC, and one large female, V (whose record is given in Table II). The normal striped specimens used (all females but one) were caught

near Ann Arbor or Detroit, except one, which came from near Cheboygan in northern Michigan. The one striped male, recorded here as "Brown," was of an unusual color and pattern, and, in fact, was not really striped. The general color was grayish fawn or taupe, and instead of stripes it had only a lateral series of occasional dark spots, giving an appearance not even resembling that of a normal garter snake. It was collected by Miss Lena M. Jessman at Ecorse (near Detroit); later another male of the same color pattern was received from the same place. Though this snake was not of usual color and pattern it was normal in respect to the factor for blackness. No snakes of normal pattern from the regions where blackness commonly occurs have been used in this work, for they might carry a recessive factor.

In brief the findings are as follows:

1. In the progenies of about fifty observed matings of striped garter snakes from the vicinities of Ann Arbor and Cheboygan, Michigan, no black ones have appeared.

2. A single mating between two black individuals, both from near Toledo, produced a brood of twenty-three young, all black.

3. Ten crosses between black and striped snakes (nine crosses were striped ♀ × black ♂; one was black ♀ × striped ♂) all gave progenies entirely normal in color, showing that black is not dominant.

4. An $F_2$ generation (from the $F_1$ from striped ♀ × black ♂) consisted of six young, of which four were normal and two black — which is as close to the 3 : 1 ratio of simple Mendelian segregation as can be obtained from six individuals.

5. Two backcrosses (black ♀ × striped hybrid ♂ of the $F_1$ generation) both gave mixed broods, one of nine striped and twelve black, the other of twenty-six striped and twenty-two black. These ratios (9 : 12, 26 : 22, total 35 : 34) are satisfactorily close to the Mendelian expectation of 1 : 1 in a backcross to the recessive parent.

Table III summarizes the successful crosses involving black and hybrid individuals.

Evidently the three black snakes from Toledo were of the same constitution in respect to melanism, for had they not had the same factor for black the matings between them would have given striped young.

TABLE III

A Record of Crosses in the Years 1931–37, Inclusive, in a Successful Attempt to Discover the Manner of Inheritance of Melanism in the Black Garter Snakes Found near Toledo

| Cross | Year | Female* | Male* | No. of young | Color of young |
|---|---|---|---|---|---|
| Black ♀ × black ♂ | 1933 | V | TC | 23 | black |
| Black ♀ × striped ♂ | 1935 | V | Brown | 43 | striped |
| Striped ♀ × black ♂ | 1931 | A | TB | 30 | striped |
| | 1933 | A | TB | 50 | striped |
| | 1931 | B | TB | 24 (?) | striped |
| | 1932 | R | TB | 23 | striped |
| | 1933 | R | TB | 28 | striped |
| | 1933 | W | TB | 38 | striped |
| | 1936 | B | TC | 44 | striped |
| | 1934 | 7,8–0 | TC | 22 | striped |
| Striped hybrid ♀ × striped hybrid ♂ | 1936 | 0–2,7,19 | 0–2,7,16 | 6 | 4 striped, 2 black (3 : 1 ratio) |
| Black ♀ × striped hybrid ♂ | 1937 | V | 2–1 | 48 | 26 striped, 22 black |
| | 1937 | 0–2,3,16 | 2–1 | 21 | 9 striped, 12 black (1 : 1 ratio) |

* The snakes referred to and their origins are as follows:

*Normal*

A, B, W } caught near Ann Arbor
Brown, from near Detroit
7,8–0, from near Detroit (?)
R, from near Cheboygan

*Black*

V, TB, TC } from Toledo
0–2,3,16, offspring of V × TC, 1933

*Hybrid, striped*

0–2,7,19, 0–2,7,16 } offspring of W × TB, 1933
2–1, offspring of A × TB, 1931

From these recorded crosses it is concluded that the melanism of the black garter snakes in the vicinity of Toledo depends on a single Mendelian recessive factor.

### IDENTITY OF THE FACTOR FOR BLACK IN THE TWO LOCALITIES

In order to find out whether the factor for black in the snakes from Ontario is the same as that in the Toledo snakes, several matings were made in the spring of 1938. Unfortunately, three

matings of a black male from Ontario failed to give young, evidently because of some incapacity of the male, and the only cross to result in a brood in August was that of a black female from Ontario and a striped male, one of whose parents was a black snake from Toledo. If these two carried the same factor the young should be black and normal in equal numbers. If they had different recessive factors for black the young should all be striped, for each parent would carry a normal factor to dominate the recessive factor for black in the other parent. The brood which was born gave a perfect 1 : 1 ratio — twelve normal, twelve black — showing that if both factors are recessive they are one and the same factor.

But unfortunately the Ontario factor has not yet been shown to be recessive. If it is dominant, and the female used in the cross under discussion is a pure dominant, all the young should be black; if, however, she is a heterozygous dominant the young should be black and normal in equal numbers, as they were.

Thus we are left with two possibilities — that the factor for black in Ontario is dominant, which seems unlikely, as the factors at Toledo and Ann Arbor are recessive, or that it is recessive and the same as that at Toledo, which seems far more probable.

### BROODS FROM WILD BLACK FEMALES COLLECTED AFTER MATING

Logier has several times (1929, 1930) reported on broods from black garter snakes taken in Ontario on the north shore of Lake Erie at Point Pelee and at Long Point, where garter snakes were "very abundant and melanism exceedingly prevalent" (1929). The broods which he obtained from females caught after their natural mating do not show simple Mendelian ratios, as do those from controlled matings.

If the black factor of the Ontario snakes is the same as that of the Toledo snakes the black females which he caught would be expected to have broods all black if the mate had been a black male, all striped if the mate had been a homozygous striped male, or black and striped in equal numbers if the mate had been a heterozygous striped male. But instead they gave such ratios of striped to black as 20 : 4, 21 : 13, 13 : 13, 5 : 15, 2 : 10, and 1 : 13.

Two records of broods from collected black females of Toledo have been given us by Mr. Conant, with the following ratios of striped to black: 12 : 4, 10 : 4.

These ratios do not match those resulting from controlled matings and would be hard to explain in a Mendelian manner. The findings from wild collected females suggest that the Mendelian ratio is obscured in some way. A possibility which suggests itself is that more than one male parent is represented in the brood. There are several ways in which this might come about. Might not a brood be contaminated by a previous mating, by some sperm being carried over for a year in the female? Might not a female copulate with two males at once? Might she not mate twice in the spring, or in the autumn and then again in the spring?

It is thinkable that the appearance of a few striped young in a brood otherwise all black might be the result of contamination from the mating of the previous spring; that sperm might be carried over a season in the female and so contaminate the next year's brood; that a black female bearing a striped brood fathered by a striped male one year might, the next year, mating with a black male, produce a black brood in which occurred a few striped individuals whose parent was the striped male of the previous season. But our breeding experiments give no evidence of this. We have in fact observed the very series of matings suggested here (see Table II): a black female bore an entirely striped brood by a striped male; the next year she bore an entirely black brood by a black male, and the next year again a striped brood by a striped male. Two chances thus existed for the appearance of contamination, if such a thing can be, by the survival of sperm from a previous spring mating; but no contamination resulted. Furthermore, a female kept over a season without mating does not have young. There has been no evidence of sperm living in the female from one spring to the next. A score of females which produced young one summer have in the next summer, without mating, produced none. About half of these females had mated in the wild and produced the young in captivity, but the remaining half were known to have mated in the spring preceding the birth.

That mating with two males simultaneously might occur and be responsible for an irregular ratio of black and striped young was shown by an incident noted in the spring of 1938. Ordinarily, in order to control the matings, only one male at a time has been placed with the females. But one day when the weather was cool enough to make it doubtful whether working with the snakes would be worth while, two males were put into a pit with two females for a speedy

test. At once both males became very active, and presently both were in actual copulation with one female. This lasted for a few minutes. Though one male was forced off, or voluntarily dropped away, before his mating could have been effectual, the incident did suggest a simultaneous double mating as a possible cause of apparently irregular ratios of normal and black young in the broods of collected females.

That effective mating may happen in the autumn is known; but we have no evidence as to whether or not a female which mated in the autumn will copulate again in the spring. If this should occur we should expect to get all kinds of irregular ratios in the resulting broods — though of course the character would nevertheless be following simple Mendelian inheritance.

Two separate spring matings of a female (the subject discussed in the following paragraphs), simultaneous mating with two males, and autumn mating (which it is now known may be effectual) followed by a second impregnation in the spring (an occurrence of which there is no evidence as yet available) are the explanations here offered for the irregular ratios of striped and black young recorded by Logier.

### SECOND MATINGS

There is now (1939) evidence that two separate spring matings may precede a brood, though from the work of the earlier years recorded in this paper it was concluded that this does not happen. Frequently in this earlier work a male had been placed in a pit where there was a female which had recently mated as well as the one with which it was desired that he should mate; thus there existed a chance for a second copulation by a female. But because of the genetical side of the study a second mating was undesirable and was somewhat guarded against, possibly at the expense of behavior observations. It always happened, when such a chance did occur, that it was the unmated female which was courted. Only once was there an apparent exception — when a female which had been disturbed during copulation and had separated from the male after only five minutes did again copulate, a week later. According to our observations and records the first copulation presumably had not reached the point of impregnation. It seemed that a female once impregnated did not copulate again.

In 1939 there was an attempt to learn more about this in order

to explain the broods of the wild black Ontario snakes. On a day in April when conditions were very favorable and the snakes especially alert and energetic a male had been put into a pit with two females, one of which had mated nineteen days before (on April 4). It was expected that he would ignore this one, but he chanced to find her first, started courtship at once, and very quickly was in copulation. For the sake of genetical interests he was at once removed; but because of the importance of the incident in connection with understanding the irregular ratios from wild black females opportunities were made for a repetition of the occurrence. On April 27 (four days later) this female again had a chance to mate, when three males were placed in the pit housing her and two other females; and very soon she was again in copulation. This spring two more such double matings occurred, after intervals of fourteen and six days.

It is important to note, however, that in each of the three instances there is some doubt as to the effectiveness of the first mating. The female discussed above produced no young in the summer even though twice in copulation of sufficient duration. The male with which she first mated (April 4) produced no young from a mating with another female, on April 22, so it seems likely that he was not a possible parent, and there may have been no insemination preceding her second copulation; and the second copulation was interrupted before there could have been insemination, so none may have preceded the third mating. Thus it is not shown by this instance that a female will mate again after one successful mating and so produce a mixed brood, from two males.

In the second instance of repeated mating a black female from Ontario mated with a black male from the same region on April 13, the attachment lasting for seventeen minutes, about an average time. Two weeks later (April 27) she mated again, this time with a striped male from near Ann Arbor, the attachment lasting for ten minutes, which has often been shown to be a sufficient period. This is the very combination suggested to account for the irregular broods from natural matings in Ontario. And there was indeed such a mixed brood borne by this female: on August 8 a progeny of sixteen was found (two living, fourteen dead in their membranes), probably born August 6. The living young were striped, but of the fourteen which did not escape from the membrane, two were unquestionably

black, and another, already partly destroyed, was presumably black. It must be assumed that the striped male from near Ann Arbor does not carry a recessive factor for blackness, as no striped snake from this region has ever been found to carry it. If the factor for black is recessive in the Ontario snakes the first mating, of the two blacks, should have produced black young, and the second mating, with the striped male, should have produced striped young. The brood should therefore be mixed, the ratio of the two types depending only on the number of functioning sperm from each male. The young accorded with this expectation. If the black factor is recessive, both males must have been represented in the progeny.

But there are two disturbing considerations. First, in the Ontario snakes the factor for black has not yet been shown to be recessive, and if it is dominant the young from the first mating, black by black, alone might give a mixed brood (though the black young would be expected to predominate or to about equal the striped). Second, the black male, which was caught in 1937, mated readily in 1938 and 1939 but produced no young, though the females with which he mated were all presumably fertile, for they had young in 1937. It would seem strange that there should be two or three of his young in this brood when his several other matings entirely failed to give progeny. When this factor for black has been shown to be recessive, however, as is that in the Toledo snakes, there will be no possible conclusion but that this mixed brood was the offspring of two males and that the female did copulate a second time after at least a slight insemination. It should be added, though, that since there were not more than three young of the first mating, it may be that the female did not then receive the usual quantity or quality of seminal fluid.

In the third instance of two matings by a female (with an interval of six days between), only eight young were born, an exceedingly small brood for a female of size to bear thirty or forty or even fifty young, indicating that the first insemination may not have been adequate for a full brood even assuming that it alone was responsible for the progeny, as there is reason, not here presented, to believe.

It has been shown, then, that under certain conditions a female will mate a second time. But as in each recorded instance of such second mating the adequacy of the insemination, if any, at the first copulation is questionable, and as there are many observations of an already mated female (which later bore a brood of normal number)

evidently evading a second courtship, it cannot now be said that after one complete impregnation a female may mate again. Perhaps it will be found that two normal inseminations may precede a brood, and both males be well represented in the brood. But perhaps a second copulation is more likely if (or occurs only if) the first was interrupted before insemination was complete, or took place in the autumn, or involved a young male or one for some reason sterile or not fully fertile.

Though it has not here been proved that any brood following two matings actually represented two male parents, there is, in two of the three instances, good reason to believe that the first male was represented, though by only a few young. The possibility of this happening often is thought evident enough to warrant suggesting it as the explanation of the odd ratios of striped and black young in the broods of collected females at Long Point.

## SUMMARY

1. Melanistic garter snakes, coal-black with pure white throats, have been found at several places on the north and west shores of Lake Erie — in Ontario at Point Pelee and Long Point (where, according to Logier, about one third of the population is black) and at various places in the vicinity of Toledo, where Conant has seen about a score.

2. An attempt to produce an $F_2$ generation by hybridizing black and normally colored (striped) garter snakes, which started in 1922 with a black male caught near Ann Arbor, was unsuccessful. The broods of two normal females caught in the autumn and confined with this male until birth of their young the following summer failed to transmit the black character. Apparently these females were already inseminated when they were caught in the autumn, and their broods of the following summer were not offspring of the black male.

3. This assumption of effective autumn mating has since been tested with females caught in the autumn and not allowed to mate the following spring. Several such females have borne young at the usual time.

4. A second attempt to study the inheritance of melanism with several specimens from Toledo showed that blackness is inherited in simple Mendelian fashion (the factor for black being recessive) as follows:

| | $F_1$ | $F_2$ |
|---|---|---|
| Striped ♀ × striped ♂ ⟶ | striped | |
| Black ♀ × black ♂ ⟶ | black | |
| Black ♀ × striped ♂ or Striped ♀ × black ♂ ⟶ | striped | ⟶ 3 striped : 1 black |
| Black ♀ × $F_1$ striped ♂ ⟶ | 1 striped : 1 black | |

5. If the factor for black in the Ontario snakes, also, is recessive (as is probable, since it is so in both the Ann Arbor snake and those from Toledo), it is the same factor that occurs in the Toledo snakes, for a cross between a black female from Ontario and a Toledo hybrid male produced a brood giving a 1 : 1 ratio.

6. Broods from Ontario females mated in the wild show very irregular ratios of striped and black (ranging from 20 : 4 to 1 : 13), which cannot be accepted as purely Mendelian. These irregular ratios suggest the possibility that more than one male is represented in the brood. This might be brought about by: autumn mating (now known to be effective) followed by a second impregnation in the spring (which has not yet been observed); copulation with two males simultaneously (which has been seen); and two separate spring matings (observed several times in 1939 though not before). None of these occurrences has yet, however, been clearly demonstrated to produce a progeny representing two male parents. Contamination of a brood by the mating for the previous brood is by experimental evidence ruled out as a probability.

For genetical studies and behavior observations of snakes of this kind it is evidently quite necessary to start with animals which have been under control for a year. Unless the history of the preceding late summer and autumn is known the spring records are useless.

A genetical understanding of the broods occurring in nature will require more studies of mating procedure and further testing of factors. One thing, however, is now clear: the factor for blackness of the Toledo garter snakes is a simple Mendelian recessive. In the field of genetics another demonstration of Mendelian inheritance is not in itself noteworthy; but in herpetology a demonstration of Mendelian inheritance is a novelty.

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# THE SENOCULIDAE OF PANAMA

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UP TO the present time only three species of Senoculidae have been recognized from Panama: *Senoculus canaliculatus* F. Cambridge, *S. purpureus* (Simon), and *S. valentinei* Petrunkevitch. Banks (1929) found no Senoculidae in his collection of 1924 from Panama. Of the forty-four mature individuals now in my possession six were taken in 1934, nineteen in 1936, and nineteen in 1939. By far the most common species found in Panama appears to be *S. canaliculatus* F. Cambridge. Cambridge gave no written description but depended entirely upon a good figure of the epigynum and an entirely inadequate figure of a part of the palpal tarsus. It appears unlikely, however, that females in my collection having epigyna so closely resembling the one figured by Cambridge can be anything but members of the same species. In spite of the inadequacy of Cambridge's figure of the male palpus it seems most probable that the males associated with females which I have regarded as identical with *S. canaliculatus* also belong to that species. Moreover, my males agree well with the description of *S. valentinei* Petrunkevitch. My present conclusion is, therefore, that *S. valentinei* is a synonym of *S. canaliculatus* F. Cambridge, and it is so treated in this paper. In view of the lack of a carefully prepared account of the female of this species I have given here a complete description, just as with new species. Unfortunately, *S. purpureus* (Simon) is not now identifiable; it must, therefore, be omitted for the present from my keys and is not again referred to in this paper. Five new species are recognized, as follows: *Senoculus barroanus*, sp. nov.; *S. bucolicus*, sp. nov.; *S. robustus*, sp. nov.; *S. silvaticus*, sp. nov.; *S. tigrinus*, sp. nov.

Knowledge concerning life histories, habits, and behavior of the Senoculidae is very meager indeed. F. Cambridge (1897–1905) wrote as follows: "The spiders themselves have the habit of lying quite flat on the bark of trees, moving with great rapidity, being remarkably Sparassiform in appearance, and they make an elongate

silken egg-cocoon." I have found these spiders on the bark of trees, but more commonly have shaken them from saplings, bushes, and vines. The first *Senoculus* I ever saw was a female adhering to a branch of a bush beside the Snyder-Molino Trail on Barro Colorado Island. She was covered by many dozens of recently hatched spiderlings, which were clinging to her body in the manner of the young in the Lycosidae. Several times I have seen females with egg cocoons fastened to a partly rolled dead leaf that was suspended by one or two threads six or eight inches long. From observations made on captive specimens it seems probable that the usual behavior for the female ready to deposit eggs is to lay down three or four short cross threads between twigs and then to hang a dead, loosely curled leaf by one thread several inches long. The eggs are laid in an oval mass on the under surface of the leaf and covered over by a mat of silken threads. The mother remains to guard the eggs and also, later, the spiderlings for a few days. She usually flattens herself over the cocoon with the first two pairs of legs outstretched in front and the two posterior pairs extended behind. Attitude and coloration thus combine to give the animal and its cocoon excellent concealment.

Methods of measuring and describing new species treated in this paper are essentially the same as those used in the author's earlier publications on Panamanian spiders (Chickering 1937a, 1937b, 1940).

Acknowledgments are again gratefully made to the directors of the Horace H. and Mary A. Rackham Fund, through whose generous support of my work the study and collection of spiders in Panama during the summers of 1934, 1936, and 1939 as well as the publication of numerous recent papers has been possible, and to Mr. Nathan Banks and Miss Elizabeth B. Bryant, both of the Museum of Comparative Zoölogy at Harvard College, who have frequently extended the courtesies of their laboratories to me. Without the help of these supporters and friends my work of recent years would have been greatly retarded.

## KEY TO THE SENOCULIDAE OF PANAMA

### MALES

1. Distinct retrolateral spine at base of cymbium; distinct beaklike process on retrolateral side of palpal bulb; prominent retrolateral finger-like process on palpal patella ..... *Senoculus bucolicus*, p. 203
1. Palpal structures not as given above for *S. bucolicus* ..... 2

2. Viewed ventrally palpal tibia wider than long; not laterally compressed; with three retrolateral cusps surrounding a pouch *S. canaliculatus*, p. 207
2. Viewed ventrally total width of palpal tibia about equal to total length; laterally compressed; with two retrolateral processes, one of which is beaklike ..... *S. barroanus*, p. 197

FEMALES

1. Main chitinized parts of epigynum distinctly converging anteriorly toward mid-ventral line ..... 2
1. Main chitinized parts of epigynum distinctly diverging anteriorly away from mid-ventral line ..... 4

2. Main chitinized lobes of epigynum extended anteriorly in slender projections; first and second tibiae with about nine pairs of ventral spines (some irregularity in this feature) ..... *S. barroanus*, p. 197
2. Main chitinized lobes of epigynum not slender anteriorly; first and second tibiae with either four pairs or about six pairs of ventral spines (some irregularity in this feature) ..... 3

3. Entire chitinized epigynal lobes somewhat diverging anteriorly; each lobe much longer than wide at base; first and second tibiae with four pairs of ventral spines ..... *S. canaliculatus*, p. 207
3. Entire chitinized epigynal lobes nearly parallel or slightly converging anteriorly; each lobe about as wide as long; first and second tibiae with about six pairs of ventral spines ..... *S. bucolicus*, p. 203

4. Each main epigynal lobe broadly extended laterally at right angle anteriorly; about as wide as long anteriorly; obliquely striated along medial half of each lobe ..... *S. silvaticus*, p. 213
4. Each main epigynal lobe not broadly extended laterally at right angle anteriorly; longer than wide anteriorly; not obliquely striated along medial half of each lobe ..... 5

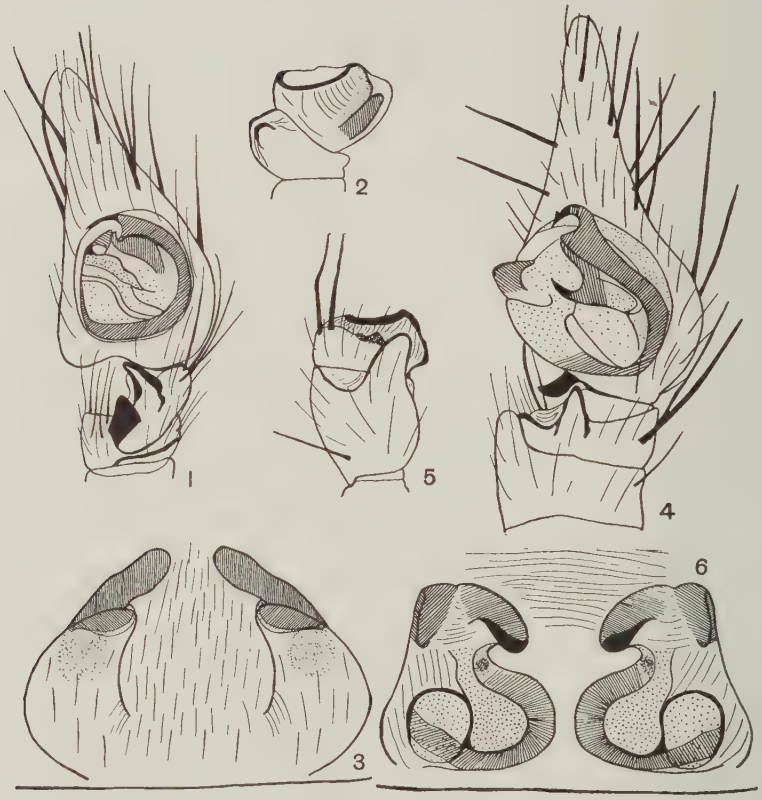
5. Each main epigynal lobe with two lobules posterior to main lobe and one broad anterior lobule ..... *S. robustus*, p. 210
5. Each main epigynal lobe with but one lobule posterior to main lobe and none anterior to it ..... *S. tigrinus*, p. 216

## Genus SENOCULUS Taczanowski, 1872

### **Senoculus barroanus**, sp. nov.

(Figures 1–3)

*Male holotype.* — Total length (body somewhat mutilated) 9.3 mm. Carapace 3.19 mm. long, 1.84 mm. wide opposite second coxae, narrowed (more gradually than is usual in this genus) to 1.41 mm. at level of LE, which project beyond margins of cephalothorax; anterior margin almost straight; moderate covering of short procumbent hair; mixture of brownish and white hair around




EXTERNAL GENITALIA OF SPIDERS

(Figures 1–6)

1. *Senoculus barroanus*, sp. nov., male palpus, ventral view
2. *S. barroanus*, sp. nov., male palpus, patella and tibia, retrolateral view
3. *S. barroanus*, sp. nov., epigynum
4. *S. bucolicus*, sp. nov., male palpus, ventral view
5. *S. bucolicus*, sp. nov., male palpus, patella and tibia, retrolateral view
6. *S. bucolicus*, sp. nov., epigynum

eyes; black bristles along margin everywhere except posterior part contiguous to abdomen; prominent tuft of yellowish hair at each anterolateral angle; prominent median longitudinal thoracic furrow; very low and flat from PME posteriorly.

*Eyes.* — Both rows very strongly recurved, ALE close to PLE. Anterior row almost as wide as posterior row. Ratio of eyes AME : ALE : PME : PLE = 7 : 3 : 8 : 8. AME separated from one another by five sevenths of their diameter, from ALE by twenty-three sevenths of their diameter. PME separated from one another by nine eighths of their diameter, from PLE by eighteen eighths of their diameter. Laterals separated by nine sixteenths of diameter of PLE. Central ocular quadrangle wider behind than in front in ratio of 24 : 16, very slightly longer than wide behind. AME on slightly raised tubercle; LE on more conspicuously raised tubercle. ALE pearly white, irregularly oval; all others dark and circular. Width of clypeus equal to radius of AME.

*Chelicerae.* — Not produced, parallel, of moderate size and robustness, not gibbous in front; basal segment 0.86 mm. long; well-developed boss. Fang of moderate size, evenly curved, very finely dentelated along inner margin. Promargin of fang groove with three rather small teeth, middle one largest; retromargin with three larger teeth. Moderately well developed scopula only along promargin.

*Maxillae.* — Parallel, longer than wide in middle in ratio of 34 : 15, only slightly concave along outer margin, not dilated apically. Palp inserted into middle third. Serrula marginal and confined to anterolateral angle. Moderately well developed scopula only along anteromedial angle.

*Lip.* — Longer than wide in ratio of 26 : 14. Basal excavations extend about ten twenty-sixths of length of organ, which reaches two thirds of length of maxillae. Anterior end somewhat narrowed so that whole lip is less rectangular than usual. Nearly straight anterior margin, provided with a group of stiff converging bristles. Sternal suture straight.

*Sternum.* — Longer than wide in ratio of 28 : 17, widest at interval between second and third coxae, considerably narrowed anteriorly and then widened a little contiguous to lip, terminating posteriorly in a sharp point that is not extended between fourth coxae, which are separated by one tenth of their diameter.

*Legs.* — 1243. Tibial index of first leg 7, of fourth leg 9.

| | *Femora* | *Patellae and tibiae* | *Metatarsi* | *Tarsi* | *Totals* |
|---|---|---|---|---|---|
| | (All measurements in millimeters) | | | | |
| 1. | 4.78 | 6.49 | 4.72 | 1.50 | 17.49 |
| 2. | 4.04 | 5.51 | 4.04 | 1.42 | 15.01 |
| 3. | 1.96 | 2.08 | 1.62 | 0.86 | 6.52 |
| 4. | 4.17 | 4.41 | 3.41 | 1.28 | 13.27 |
| *Palp* | 0.92 | 0.25 + 0.23 | ... | 1.23 | 2.63 |

Ventral spines of first and second tibiae 2 – 2 – 2 – 2 – 2 – 2 – 2 – 2 – 2 – 0; of third tibiae 1p – 1p – 1p; of fourth tibiae 1p – 2 – 0 (there is some irregularity among these). Ventral spines of first and second metatarsi, seven along prolateral side and eight along retrolateral side (a cursory examination would probably result in recording eight pairs); of third metatarsi 2 – 1p – 1p; of fourth metatarsi 2 – 2 – 1r. Ventral spines of first and second legs very long and robust, as usual in this genus. Trichobothria observed as follows: only three or four on tarsi, several irregularly placed on metatarsi, and many on tibiae, all on dorsal sides of segments named. Three claws throughout; proclaw with eight or nine slender teeth, proximal two or three very slender and crowded; retroclaw with six or seven slender teeth. All legs have many spines not recorded here and all have fairly complete coating of short brown hair and scanty supply of long light hair. Fourth legs and, to a lesser extent, third legs with ventral brushes of hair on metatarsi and distal halves of tibiae.

*Palp.* — Both patella and tibia very short, the former slightly longer than the latter. Patella wider than long, with very short, retrolateral nodule representing apophysis, which appears in certain species. Tibia compressed and somewhat extended ventrally, where there are low ridges and eminences. Also a shallow retrolateral excavation (Figs. 1–2).

*Abdomen.* — Longer than wide in ratio of 54 : 10, widest at base, diminishing gradually to posterior end; only slightly flattened. Anal tubercle a flattened cone. Spinnerets as usual in genus. Impossible to see colulus or tracheal spiracle because of mutilation.

*Color in alcohol.* — Yellow ground color of various shades, under parts all lighter. Cephalothorax, chelicerae, and legs dorsally light amber. Cephalothorax with black around eyes and broad dusky brown margin. Dorsum of abdomen light yellow with several very narrow irregular dark stripes along each lateral side; elongated dark spots dorsally. Legs with dark irregular dorsal blotches.

*Female allotype.* — Total length 10.41 mm. Carapace 3.31 mm. long, 2.21 mm. wide opposite second coxae, 1.65 mm. wide at level of PLE, which project slightly beyond margin; anterior border nearly straight; short brown hair either sparsely developed or worn off in handling; a few white hairs in region of eyes; longitudinal thoracic groove a shallow basin; low and flat throughout.

*Eyes.* — Both rows of eyes very strongly recurved, AME close to anterior margin, ALE very small and close to PLE. Two rows almost equal in length. Ratio of eyes AME : ALE : PME : PLE = 5 : 2.5 : 7.5 : 8. AME separated from one another by six fifths of their diameter, from ALE by twenty-eight fifths of their diameter. PME separated from one another by twenty-one fifteenths of their diameter, from PLE by forty-five fifteenths of their diameter. Laterals separated by a little more than one half of diameter of PLE. Central ocular quadrangle wider behind than in front in ratio of 5 : 3, as long as wide behind. AME on low eminence; LE on common tubercle, with small ALE clinging to anterior border. ALE pearly white, irregularly oval; all others dark, regularly circular. Height of clypeus equal to about four fifths of diameter of AME.

*Chelicerae.* — Not produced, fairly robust, somewhat gibbous in front near base; basal segment 1.10 mm. long; well-developed boss. Fang of moderate size, evenly curved. Promargin of fang groove with three teeth, middle one largest (only two teeth on left side); retromargin with three larger teeth close together, middle one slender, others more robust. Well-developed scopula only along promargin.

*Maxillae.* — Very slightly divergent, longer than wide in middle in ratio of 40 : 16, only slightly concave along outer border, slightly dilated apically. Palp inserted between basal and middle third. Marginal serrula extending from scopula over apical outer angle. Scopula confined to apical medial angle.

*Lip.* — Longer than wide in ratio of 27 : 15. Basal excavations extend about twelve twenty-sevenths of length of organ, which reaches about five eighths of length of maxillae. Considerably narrowed in apical third; slightly or not at all notched apically. About ten or twelve stiff black converging apical marginal bristles. Sternal suture straight.

*Sternum.* — Longer than wide in ratio of 29 : 17, widest between second coxae, narrowed to 10 units (as compared to 17) between

first coxae, and then apparently widened again and extended to meet lip at sternal suture.

*Legs.* — 1243. Tibial index of first leg 10, of fourth leg 10. Legs somewhat mutilated.

| | *Femora* | *Patellae and tibiae* | *Metatarsi* | *Tarsi* | *Totals* |
|---|---|---|---|---|---|
| | | (All measurements in millimeters) | | | |
| 1. | 4.78 | 5.51 | 4.04 | 0.98 | 15.31 |
| 2. | 4.04 | 5.14 | 3.37 | 1.00 | 13.55 |
| 3. | 2.21 | 2.19 | 1.41 | 0.90 | 6.71 |
| 4. | 4.29 | 4.29 | 2.82 | 1.10 | 12.50 |

Ventral spines of first tibiae 2 – 2 – 2 – 2 – 2 – 2 – 2 – 2 – 2 – 0 (last pair replaced by pair of terminal bristles); of second tibiae, nine prolateral and eight retrolateral spines, not all definitely paired (also with pair of bristles replacing terminal pair of spines); of third tibiae 1p – 1p – terminal prolateral bristle and a row of retrolateral spines suggesting a comb for some special purpose; of fourth tibiae 0 – 1p – row of five stiff terminal bristles (weak spines). Ventral spines of first and second metatarsi, seven prolateral and eight retrolateral, not all definitely paired and none terminal (difficult here to distinguish between lateral and ventral spines); of third metatarsi 2 – 1p – 1p; of fourth metatarsi 2 – 2 – 0. Ventral spines of first and second legs very long and robust. Tarsus and tibia of palp very spiny; palpal claw with several short teeth. Trichobothria apparently distributed as in male but exact determination difficult because many seem broken off. Three claws throughout; proclaw and retroclaw probably with nine teeth in a single row. Legs with little hair, but some of original coating may have been worn off. Fourth tarsi and metatarsi with moderately well developed brushes.

*Abdomen.* — Longer than wide in ratio of 58 : 14, widest about one tenth of distance from base; somewhat distorted but probably considerably flattened in life. Anal tubercle a flattened cone. Spinnerets normal to genus. Colulus and tracheal spiracle not visible because of distortion.

*Epigynum.* — Considerably different from that of any other species I have studied. Most characteristic feature is presence of two slender anterior converging extensions of chitinized lobes (Fig. 3).

*Color in alcohol.* — Somewhat lighter than male. Cephalothorax with only narrow dusky brown margin. Abdomen without irregu-

lar dark stripes along lateral sides and with only a few dark spots on dorsum and none on legs dorsally.

*Type locality.* — Male holotype from Canal Zone Forest Reservation, Canal Zone, Aug., 1936. Female allotype from Gamboa, Canal Zone, Aug., 1939. Male paratypes from Barro Colorado Island, Canal Zone, July, 1934, and Aug., 1939, Canal Zone Forest Reservation, Canal Zone, Aug., 1939.

### **Senoculus bucolicus,** sp. nov.

(Figures 4–6)

*Male holotype.* — Total length (from clypeus to posterior end of anal tubercle) 8.21 mm. Carapace 3.19 mm. long, widest opposite second coxae, where it is 2.70 mm. wide, sharply narrowed to 1.47 mm. at level of PLE, which occupy almost full width of head at that level; covered with moderately well developed coat of brownish procumbent hairs; white hairs around eyes; deep longitudinal thoracic furrow; very flat, much depressed in region of thoracic furrow.

*Eyes.* — Both rows of eyes strongly recurved and very characteristic of genus. Posterior row longer than anterior row in ratio of 55 : 49. Ratio of eyes AME : ALE : PME : PLE = 6 : 4.5 : 11 : 11. AME separated from one another by five sixths of their diameter, from ALE by nineteen sixths of their diameter. PME separated from one another by nine elevenths of their diameter, from PLE by twelve elevenths of their diameter. Laterals separated by six elevenths of diameter of PLE. Central ocular quadrangle wider behind than in front in ratio of 5 : 3, wider behind than long in ratio of 25 : 24. AME on slightly raised tubercle; LE on somewhat more prominent confluent tubercle. ALE pearly white; all others dark. Height of clypeus equal to five sixths of diameter of AME.

*Chelicerae.* — Not produced, fairly robust, parallel; basal segment 0.95 mm. long; boss well developed. Fang normal, evenly curved, rather finely dentelated along inner margin. Promargin of fang groove with three teeth, middle one largest; retromargin with three larger teeth. Moderately well developed scopula only on promargin.

*Maxillae.* — Very slightly convergent, longer than wide in ratio of 36 : 19, only slightly concave along outer margin, not dilated apically. Palp inserted into second quarter from base.

*Lip.* — Longer than wide in ratio of 23 : 15. Basal excavations reach little less than half way to distal end. Nearly rectangular. Sternal suture straight.

*Sternum.* — Longer than wide in ratio of 27 : 21, widest at interval between second and third coxae, pointed behind but not extended between fourth coxae, which are separated by one third of their diameter. Long black bristle in the middle of each coxa on ventral side.

*Legs.* — 1243. Tibial index of first leg 9, of fourth leg 11.

| | *Femora* | *Patellae and tibiae* | *Metatarsi* | *Tarsi* | *Totals* |
|---|---|---|---|---|---|
| | (All measurements in millimeters) | | | | |
| 1. | 4.41 | 5.64 | 4.53 | 1.96 | 16.54 |
| 2. | 4.04 | 5.02 | 4.29 | 1.98 | 15.33 |
| 3. | 3.68 | 3.06 | 2.70 | 1.47 | 10.91 |
| 4. | 2.70 | 3.80 | 3.70 | 1.72 | 11.92 |
| *Palp* | 1.34 | 0.26 + 0.49 | ... | 1.41 | 3.50 |

Ventral spines of first and second tibiae 2 – 2 – 1r – 2 – 2 – 2 – 0; of third tibiae 1p – 1p – 1p; of fourth tibiae 0 – 2 – 0. Ventral spines of first and second metatarsi, five prolateral and six retrolateral, with much irregularity of position; of third metatarsi 2 – 2 – 1p – 1p; of fourth metatarsi 2 – 1p – 1p – 1r – 0. Ventral spines on first and second legs very long and robust. Trichobothria distributed as follows: four pairs on tarsi, several on metatarsi, and many on tibiae. Three claws throughout; proclaw apparently with six slender teeth; retroclaw with five (?). Legs clothed with scantily arranged long hairs and considerable short brown hair. Weakly developed ventral brushes of hairs on third and fourth metatarsi and tibiae as described for *S. valentinei* Petrunkevitch.

*Palp.* — Patella and tibia very short. Patella with short blunt retrolateral apophysis. Tibia with well-chitinized low semicircular ridge on anteroventral surface and two low retrolateral processes forming shallow groove between them. Tarsus with strongly chitinized retrolateral spur at its base; distal half of tarsus long and slender. Bulb provided with several processes, as shown in Figures 4–5. Fourteen heavy spines on cymbium.

*Abdomen.* — Longer than wide in ratio of 36 : 12; rather flat (it is possible that a part of flattening is due to distortion). Anal tubercle a prominent cone. Colulus a low broad distinct cone. Anterior spinnerets robust, with their bases separated by colulus;

middle spinnerets small, contiguous; posterior spinnerets longest, with terminal segment more than half as long as basal segment. Tracheal spiracle just anterior to base of colulus.

*Color in alcohol.* — Under parts all more or less yellowish, with palpal tarsus deep amber. All eyes encircled with dark brown or black pigment with only little color around AME. Carapace yellowish. All appendages yellow dorsally. Abdomen generally darker yellowish; body wall just over heart region quite transparent and colored by organs within; almost all of dorsal abdominal area except median transparent region provided with numerous angular yellowish chalky spots. In a paratype third legs irregularly black ventrally on last three segments.

*Female allotype.* — Total length 9.19 mm. Carapace 3.51 mm. long, 3.06 mm. wide opposite second coxae, abruptly narrowed to 1.84 mm. at level of PLE, which occupy slightly more than thirteen fifteenths of width of head at that level; anterior border almost a straight line; covered with moderately heavy coat of reddish brown hair with a few black hairs intermixed and a few white ones in region of eyes; deep thoracic furrow; very low throughout and depressed in region of thoracic furrow.

*Eyes.* — Both rows of eyes very much recurved and distinctly characteristic of genus. Posterior row longer than anterior row in ratio of 30 : 27. Ratio of eyes AME : ALE : PME : PLE = 6 : 3.5 : 11 : 11. AME separated from one another by five sixths of their diameter, from ALE by twenty-five sixths of their diameter. PME separated from one another by ten elevenths of their diameter, from PLE by fourteen elevenths of their diameter. Laterals separated by five elevenths of diameter of PLE. Central ocular quadrangle wider behind than in front in ratio of 29 : 15, wider behind than long in ratio of 29 : 27. AME on low common tubercle; LE on somewhat more prominent common tubercles. ALE pearly white; all others dark. Height of clypeus equal to about five sixths of diameter of AME.

*Chelicerae.* — Fairly robust, somewhat gibbous in front, not produced, parallel; basal segment 1.35 mm. long; boss well developed. Fang rather robust, evenly curved, dentelated along inner margin. Promargin of fang groove with three teeth of moderate size; retromargin with three much larger teeth. Moderately well developed scopula only on promargin.

*Maxillae.* — Parallel, longer than wide in ratio of 2 : 1, almost straight along lateral margin, not dilated apically. Palp inserted into second quarter from base. Marginal serrula only along outer anterolateral angle. Well-developed scopula on inner anterolateral angle.

*Lip.* — Longer than wide in ratio of 32 : 20. Basal excavations reach almost exactly half of length of organ. Distal margin very slightly concave and provided with row of stiff converging bristles. Sternal suture slightly procurved.

*Sternum.* — Longer than wide in ratio of 29 : 25, widest between second coxae, pointed behind but not extended between fourth coxae, which are separated by about one third of their diameter. Long black bristle in center of third and fourth coxae (may be present on other coxae in life).

*Legs.* — 1243. Tibial index of first leg 9, of fourth leg 11.

| | *Femora* | *Patellae and tibiae* | *Metatarsi* | *Tarsi* | *Totals* |
|---|---|---|---|---|---|
| | (All measurements in millimeters) | | | | |
| 1. | 4.04 | 5.51 | 3.77 | 1.60 | 14.92 |
| 2. | 3.68 | 4.90 | 3.43 | 1.47 | 13.48 |
| 3. | 2.74 | 3.19 | 2.70 | 1.23 | 9.86 |
| 4. | 3.80 | 3.78 | 3.55 | 1.48 | 12.61 |

Ventral spines of first tibiae 2 – 2 – 1r – 2 – 2 – 2 – 0 (with an extra one on both legs between second and third on retrolateral side); of second tibiae 1p – 2 – 1r – 2 – 2 – 2 – 0 (left), 2 – 2 – 1r – 2 – 2 – 2 – very heavy bristles which might be called weak spines (right); of third tibiae 1p – 1p – 2 (weak); of fourth tibiae 0 – 1p – 2 (weak). Ventral spines of first metatarsi 2 – 2 – 1r – 2 – 2 – 2 – 1 (median); of second metatarsi 2 – 2 – 1r – 2 – 2 – 2 – 1 (median); of third metatarsi 2 – 0 – 1 (median); of fourth metatarsi 2 – 1p – 1r – 2 (small). Ventral spines of first and second legs very long and robust. Trichobothria apparently in four pairs on tarsi but numerous on metatarsi and tibiae. Three claws throughout; retroclaw with five slender teeth; proclaw with six. All legs with scantily distributed long hair and much short brown hair. Third and fourth tibiae with distinct ventral brushes; third and fourth metatarsi with weakly developed ones. Palpus with tarsal claw having four teeth.

*Abdomen.* — Longer than wide in ratio of 45 : 15; much flattened. Anal tubercle a broad flat cone with a tuft of reddish brown hair

ventral to it. Colulus obscured by wrinkles but probably present as a fairly conspicuous cone. Anterior spinnerets robust, not contiguous at their bases, which are separated by what is considered to be colulus. Middle spinnerets small, slender, obscured by others; posterior spinnerets longest, with terminal segment one half as long as basal segment; much reddish hair. Tracheal spiracle just anterior to colulus.

*Epigynum.* — With two strongly chitinized hooks whose broad bases are very close together; provided with several ridges and grooves (Fig. 6).

*Color in alcohol.* — Essentially as described for male except that ventrally third tibiae and metatarsi are almost black and third tarsi have black prolateral stripe.

*Type locality.* — Male holotype and female allotype from Barro Colorado Island, Canal Zone, July, 1936. Male paratypes from Barro Colorado Island, Canal Zone, July, 1934, 1936, 1939. Female paratypes from same locality, June–July, 1934.

### *Senoculus canaliculatus* F. Cambridge

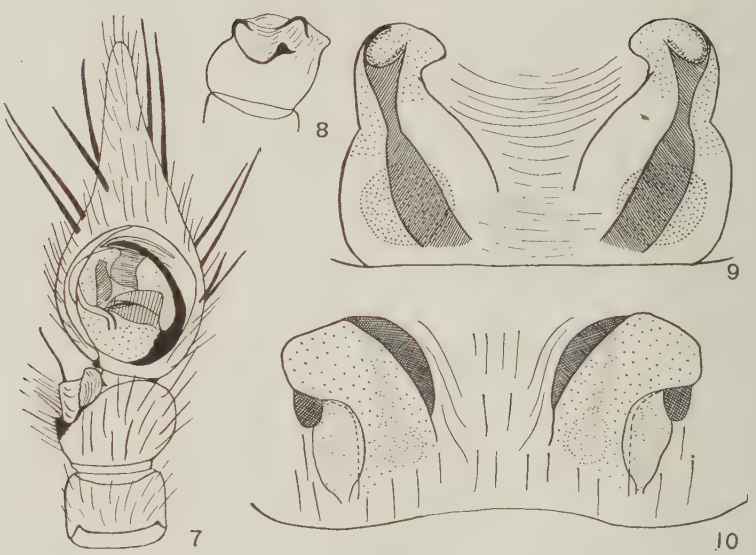
*Senoculus valentinei* Petrunkevitch, 1925.

(Figures 7–9)

In view of the lack of a careful description of the female of this species I am providing one here, just as for a new species. My figures of the male palpus and the epigynum (Figs. 7–9), also, are given here for comparison with those published by Cambridge (1897–1905) and Petrunkevitch (1925).

*Female.* — Total length 12.74 mm. Carapace 4.29 mm. long, widest opposite second coxae, where it is 3.30 mm. wide, narrowed abruptly at level of PLE to 2.08 mm.; posterior row of eyes occupy almost full width of head at that level; anterior border nearly straight; covered with moderately heavy coating of brown procumbent hair; stiff bristles along margins and especially along anterior border; white hairs in vicinity of eyes; tuft of hairs at each anterolateral angle; fairly deep longitudinal thoracic furrow; very low and flat, with head region only slightly raised.

*Eyes.* — As usual in this genus both rows of eyes very strongly recurved and ALE very small and close to PLE. Posterior row only slightly wider than anterior row. Ratio of eyes AME : ALE :




EXTERNAL GENITALIA OF SPIDERS

(Figures 7–10)

7. *Senoculus canaliculatus* F. Cambridge, male palpus, ventral view
8. *S. canaliculatus* F. Cambridge, male palpus, tibia, retrolateral view
9. *S. canaliculatus* F. Cambridge, epigynum
10. *S. robustus*, sp. nov., epigynum

PME : PLE = 6 : 4 : 11 : 11. AME separated from one another by slightly less than their diameter, from ALE by six times their diameter. PME separated from one another by slightly more than their diameter, from PLE by twenty-five elevenths of their diameter. Laterals separated by six elevenths of diameter of PLE. Central ocular quadrangle wider behind than in front in ratio of 30 : 14, almost as long as wide behind. AME on low tubercle; LE on somewhat more prominent common tubercle; ALE free from head below. ALE pearly white and oval; all others circular and dark. Width of clypeus slightly less than diameter of AME.

*Chelicerae.* — Not produced, robust, parallel, gibbous in front; basal segment 1.72 mm. long; well-developed boss. Fang fairly robust, evenly curved, dentelated along inner margin. Promargin

of fang groove with three teeth of moderate size, middle one largest; retromargin with three larger teeth. Moderately well developed scopula on promargin.

*Maxillae.* — Parallel, longer than wide in ratio of 27 : 13, slightly concave along outer border, not dilated apically. Palp inserted into second quarter from base. Marginal serrula confined to anterolateral angle. Well-developed scopula confined to anteromedial angle.

*Lip.* — Longer than wide in ratio of 43 : 21. Basal excavations extend to about middle point of organ, which reaches two thirds of length of maxillae. Almost straight across distal border; group of stiff converging distal bristles. Nearly rectangular. Sternal suture straight.

*Sternum.* — Longer than wide in ratio of 40 : 26, widest opposite interval between second and third coxae, considerably narrowed anteriorly, terminating posteriorly in a rather sharp point that is not extended between fourth coxae, which are separated by five thirteenths of their diameter.

*Legs.* — 1243. Tibial index of first leg 10, of fourth leg 12.

| | *Femora* | *Patellae and tibiae* | *Metatarsi* | *Tarsi* | *Totals* |
|---|---|---|---|---|---|
| | (All measurements in millimeters) | | | | |
| 1. | 5.00 | 6.68 | 3.92 | 1.35 | 16.95 |
| 2. | 4.41 | 5.39 | 3.31 | 1.38 | 14.49 |
| 3. | 2.82 | 3.19 | 2.48 | 1.23 | 9.72 |
| 4. | 4.29 | 3.98 | 2.94 | 1.35 | 12.56 |

Ventral spines of first and second tibiae 2 – 2 – 2 – 2 – 0; of third tibiae 1p – 1p (?) – 0; of fourth tibiae 0 – 2 (irregular) – 0. Ventral spines of first and second metatarsi 2 – 2 – 2 – 2 – 0 (ventral spines may easily be confused with laterals); of third metatarsi 2 – 1p – 2 (?); of fourth metatarsi 2 – 2 – 2. Ventral spines of first and second tibiae and metatarsi very long and robust. Trichobothria observed as follows: double row of five or six in each row on tarsi and metatarsi; numerous on dorsal side of tibiae; apparently forming fringe on prolateral surface of third femora. Three claws throughout; proclaw with seven slender teeth; retroclaw with six teeth. All legs with many spines not recorded here, and last two segments of palps with many long spines irregularly placed. Also palpal distal claw with five slender teeth. All legs with fairly thick covering of short brown hair and rather scanty supply of much longer, lighter-

colored hair. Palps quite hairy. Fourth legs with distinct ventral brushes on metatarsi and tibiae.

*Abdomen.* — Longer than wide in ratio of 7 : 3, not so slender as in most species from Panama; in this species tending to be swollen dorsally and laterally about midway. Two small rounded dorsal tubercles about one quarter of distance from base and a single larger median prominence slightly behind middle of dorsal area usual in these females but may be entirely lacking. Colulus a fairly prominent cone with tracheal spiracle just a short distance anterior to it. Spinnerets normal to genus.

*Epigynum.* — Two main lobes somewhat divergent; anterior ends directed medially (Fig. 9).

*Color in alcohol.* — Under parts all more or less yellowish. Chelicerae and palpal tarsus amber-colored. Dorsally all appendages darker yellow with brown spots in some specimens. Margin of carapace brownish. Eyes surrounded with dark brown or black pigment. Dorsally abdomen shows transparent cardiac stripe and reddish brown mottling irregularly overlying yellowish ground color. Also many small chalky whitish angular spots all over dorsal area except cardiac stripe. Brown coloration marked in a specimen from Porto Bello, and almost lacking in some taken on Barro Colorado Island.

*Collection records.* — This seems to be the most common *Senoculus* in Panama. I have specimens in my collection as follows: males — Barro Colorado Island, June, 1936, Aug., 1936 and 1939, Fort Randolph, Aug., 1936, Fort Sherman, Aug., 1939; females — Barro Colorado Island, July, 1936, June and July, 1939, Fort Davis and Porto Bello, Aug., 1936, Canal Zone Forest Reservation, July, 1939, Madden Dam, Aug., 1939.

### **Senoculus robustus,** sp. nov.

(Figure 10)

*Female holotype.* — Total length 12.50 mm. Carapace 5.15 mm. long, 4.16 mm. wide at level of second coxae, almost round behind posterior eyes, narrowed to 2.82 mm. at level of LE, which occupy twenty twenty-thirds of its width; anterior margin nearly straight, with lateral corners rounded; well-developed coat of mixed short brown and yellowish procumbent hair; white hairs on the deeply

pigmented areas around eyes; posterior three fifths of lateral margin with fringe of brown bristles and long light yellowish hair, anterior two fifths with longer light yellowish hair; longitudinal thoracic furrow short, deep, and broader than usual; low and flat, with head only slightly raised.

*Eyes.* — Both rows strongly recurved, ALE small and close to PLE. Posterior row wider than anterior row in ratio of 45 : 43. Ratio of eyes AME : ALE : PME : PLE = 5.5 : 3 : 11 : 12. AME separated from one another by about seven fifths of their diameter, from ALE by about eight times their diameter. PME separated from one another by fifteen elevenths of their diameter, from PLE by thirty elevenths of their diameter. Laterals separated by seven twelfths of diameter of PLE. Central ocular quadrangle wider behind than in front in ratio of 36 : 16, only slightly longer than wide behind. AME slightly raised and directed somewhat laterally; LE on low confluent tubercles; ALE free beneath. ALE pearly white and somewhat oval; all others circular and dark. Width of clypeus equal to about seven fifths of diameter of AME.

*Chelicerae.* — Not produced, robust, essentially parallel, gibbous in front; basal segment 1.84 mm. long; well-developed boss. Fang robust, evenly curved, finely dentelated along inner margin. Promargin of fang groove with three teeth, middle one largest, others rather small; retromargin with three teeth, outermost one somewhat more robust than other two. Quite well developed scopula only on promargin.

*Maxillae.* — Parallel, fairly robust, longer than wide in middle in ratio of 33 : 14, slightly concave along outer border, not dilated apically but somewhat pointed in middle of anterior border. Palp inserted into second quarter from base. Marginal serrula extends over outer half of anterior border. Well-developed scopula along inner half of anterior border and long curved hairs along outer margin.

*Lip.* — Longer than wide at base in ratio of 24 : 15. Basal excavations extend to middle of organ, which reaches two thirds of length of maxillae. Nearly straight across distal border; group of converging black distal bristles. Nearly rectangular. Sternal suture straight.

*Sternum.* — Longer than wide in ratio of 21 : 15, widest opposite second coxae, narrowed to one third its length between first coxae, terminating posteriorly in a sharp point that is not extended between

posterior coxae, which are separated by about one fourth of their diameter.

*Legs.* — 1243. Tibial index of first leg 11, of fourth leg 14.

| | *Femora* | *Patellae and tibiae* | *Metatarsi* | *Tarsi* | *Totals* |
|---|---|---|---|---|---|
| | | (All measurements in millimeters) | | | |
| 1. | 5.39 | 7.23 | 4.29 | 1.59 | 18.50 |
| 2. | 4.90 | 6.13 | 4.04 | 1.59 | 16.66 |
| 3. | 3.55 | 4.04 | 3.19 | 1.35 | 12.13 |
| 4. | 5.02 | 5.01 | 3.55 | 1.59 | 15.17 |

Ventral spines of first and second tibiae 2 – 2 – 2 – 2 – pair of terminal bristles; of third tibiae 1p – 1p – 4 retrolateral weak spines in a transverse row; of fourth tibiae 1p – 2 – 3 weak retrolateral spines in a transverse terminal row. Ventral spines of first and second metatarsi 2 – 2 – 2 – 0; of third metatarsi 2 – 1p – 1p; of fourth metatarsi 2 – 2 – 2. Ventral spines of first and second legs quite long and robust. Tibia and tarsus of palp very spiny. Palpal claw with about seven slender teeth increasing in length from basal tooth. Three claws throughout; proclaw with about six teeth; retroclaw with six. All legs with moderately well developed coat of short brown hairs; femora, especially of first two pairs of legs, with conspicuous long light yellowish hair giving shaggy appearance to this species.

*Abdomen.* — Longer than wide in ratio of 64 : 20; width about same to posterior end, where it is gradually narrowed to base of spinnerets; considerably flattened. Anal tubercle a flattened cone. Spinnerets normal to genus. Colulus soft and weakly developed. Small tracheal spiracle just anterior to colulus. Well-developed coat of short, light-colored hair, with short weak sparsely arranged bristles and considerable long light hair along lateral sides.

*Epigynum.* — Characterized by two massive strongly chitinized lobes, not hooklike, diverging as shown in Figure 10.

*Color in alcohol.* — Under parts generally light yellowish except lip, maxillae, and chelicerae, which are amber-colored. In front chelicerae darker amber. Dorsally legs light amber. Cephalothorax amber-colored and somewhat grayed by presence of hair. Dorsally abdomen has light-colored lancelike stripe over heart; irregular dark brown spot just behind cardiac stripe; many broken alternate light and dark stripes posteriorly and laterally.

*Type locality.* — Female holotype from Barro Colorado Island, Canal Zone, June 30, 1934. Taken with many spiderlings clinging to mother and remnants of loosely constructed cocoon in a partly curled leaf attached to bark on one of "Big Trees" on Armour Trail.

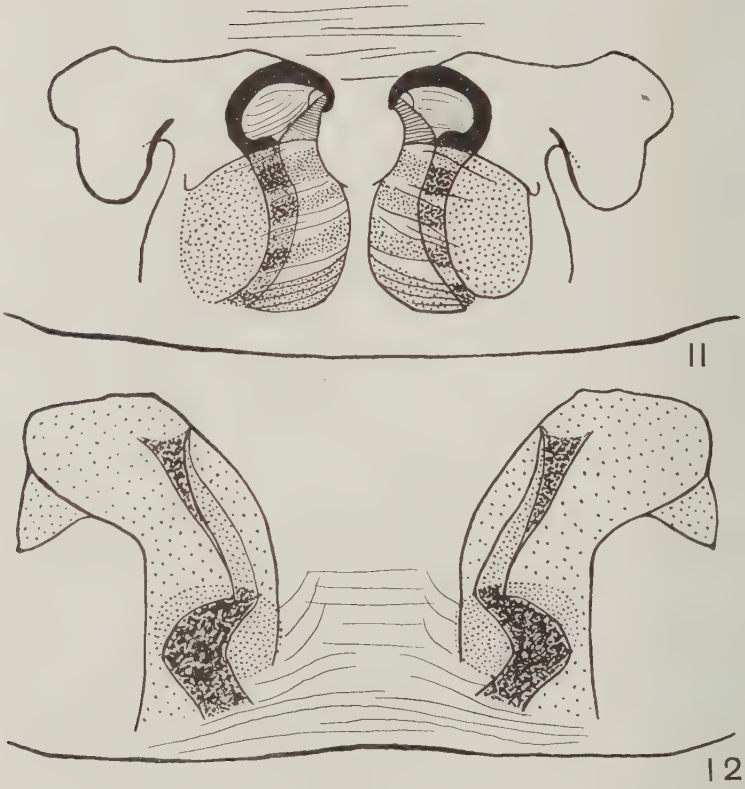
### **Senoculus silvaticus,** sp. nov.

(Figure 11)

*Female holotype.* — Total length 8.58 mm. Carapace 3.12 mm. long, 2.70 mm. wide (nearly round in outline from posterior eyes to posterior margin) at level of second coxae, narrowed to 1.65 mm. at level of LE, which occupy fourteen fifteenths of width; anterior margin nearly straight but lateral corners more rounded than is usual in this genus; moderately well developed covering of short brown procumbent hair; white hairs on pigmented areas surrounding eyes; a few short dark marginal bristles along margin except at anterior border, where they are long and slender; one long bristle just ventral to AME space; fairly deep but short longitudinal thoracic furrow; low and quite flat, with cephalic portion slightly raised.

*Eyes.* — Both rows of eyes very strongly recurved, ALE very close to PLE. Posterior row wider than anterior in ratio of 14 : 12. Ratio of eyes AME : ALE : PME : PLE = 6 : 4 : 12 : 11.5. AME separated from one another by five sixths of their diameter, from ALE by twenty sixths of their diameter. PME separated from one another by nine twelfths of their diameter, from PLE by fourteen twelfths of their diameter. Laterals separated by ten twenty-thirds of diameter of PLE. Central ocular quadrangle wider behind than in front in ratio of 28 : 14, wider behind than long in ratio of 28 : 24. AME slightly raised and directed somewhat laterally; LE on low confluent tubercles. ALE pearly white, irregularly oval; all others dark and circular. Width of clypeus equal to seven sixths of diameter of AME.

*Chelicerae.* — Not produced, parallel, of moderate size and robustness; slightly gibbous in front; basal segment 1.10 mm. long; well-developed boss. Fang moderately robust, evenly curved, finely dentelated along inner margin. Promargin of fang groove with three teeth, middle one largest; retromargin with three larger teeth more closely crowded. Moderately well developed scopula only along promargin.




EXTERNAL GENITALIA OF SPIDERS

(Figures 11–12)

11. *Senoculus silvaticus*, sp. nov., epigynum
12. *S. tigrinus*, sp. nov., epigynum

*Maxillae.* — Very slightly convergent distally, longer than wide in middle in ratio of 40 : 21, lateral margins straight, not dilated apically. Palp inserted into middle third. Marginal serrula only along lateral half of distal border and outer anterolateral angle. Moderately well developed scopula along medial half of anterior border and inner anterolateral angle.

*Lip.* — Longer than wide in ratio of 29 : 19. Basal excavations reach almost half length of organ, which extends two thirds of length of maxillae. Distal end somewhat narrowed so that whole organ is less rectangular than is frequently the case in this genus. Somewhat notched at distal end, where there is a row of converging black bristles. Sternal suture straight.

*Sternum.* — Longer than wide in ratio of 25 : 22, much rounder in outline than is usual in this genus, widest just posterior to second coxae, covered with moderately heavy coat of fine upright bristles, not much narrowed anteriorly, terminating in a blunt point that does not extend between fourth coxae, which are separated by one half their width.

*Legs.* — 1243. Tibial index of first leg 11, of fourth leg 13.

| | *Femora* | *Patellae and tibiae* | *Metatarsi* | *Tarsi* | *Totals* |
|---|---|---|---|---|---|
| | | (All measurements in millimeters) | | | |
| 1. | 3.55 | 4.17 | 3.00 | 1.47 | 12.19 |
| 2. | 3.43 | 4.04 | 2.70 | 1.38 | 11.55 |
| 3. | 2.45 | 2.45 | 2.33 | 1.19 | 8.42 |
| 4. | 3.31 | 3.19 | 2.63 | 1.35 | 10.48 |

Ventral spines of first and second tibiae 2 – 2 – 2 – 2 – 0 (terminal spines replaced by stiff bristles); of third and fourth tibiae 1p – 1p – 0 (terminal spines replaced by pair of stiff robust bristles). Ventral spines of first and second metatarsi 2 – 2 – 2 – 1 (median); of third metatarsi 2 – 2 – 1p; of fourth metatarsi 2 – 1p – 2 – 0 (difficult to distinguish between lateral and ventral spines). Ventral spines of first and second legs very long and robust. Tarsus and tibia of palp very spiny. Palpal claw with five teeth increasing in length from small basal to rather long and slender distal tooth. Trichobothria observed as follows: a few scattered on palpal tibiae; double dorsal row of ten on tarsi; fifteen to twenty dorsal on metatarsi apparently not arranged in regular rows; many on tibiae and crowded near proximal end. Three claws throughout; proclaw and retroclaw each with five or six slender teeth. All legs provided with moderately complete coat of short brown hair and sparse long light-colored hair. Fourth tibiae with rather conspicuous ventral brushes of dark hair; remnants of brushes appear on metatarsi of fourth legs and on both metatarsi and tibiae of third legs.

*Abdomen.* — Longer than wide in ratio of 43 : 21, widest at about

beginning of last third, nearly cylindrical at base; somewhat flattened posteriorly. Anal tubercle with bulbous base and conical distal part. Spinnerets normal to genus. Colulus a low, soft, much-reduced cone. Tracheal spiracle just anterior to colulus.

*Epigynum.* — Two lobes intricately sculptured into ridges, grooves, and processes, as shown in Figure 11.

*Color in alcohol.* — Essentially as described for *S. barroanus*, sp. nov., except that dorsum of abdomen has considerable light reddish brown color irregularly distributed over it.

*Type locality.* — Female holotype from Barro Colorado Island, Canal Zone, July, 1936. Female paratype from same locality, June, 1939.

### **Senoculus tigrinus,** sp. nov.

(Figure 12)

*Female holotype.* — Total length 15.07 mm. Carapace 6.15 mm. long, 4.90 mm. wide opposite second coxae, nearly round in outline posterior to PE, where carapace measures 3.06 mm. wide; posterior eyes occupy twelve thirteenths of width of head at that level; anterior margin almost straight, with tuft of yellowish hairs at each lateral corner; covering of short brown procumbent hair; numerous brown bristles along all margins except posterior margin next to abdomen, where there is a band of short white hairs; numerous white hairs in vicinity of pigmented areas surrounding eyes; long bristle just ventral to AME space; usual median longitudinal thoracic furrow apparently lacking in this species but instead a fairly deep rounded pit containing two muscle impressions; low and quite flat, with cephalic part only raised a little.

*Eyes.* — Both rows very strongly recurved, ALE close to PLE. Posterior row wider than anterior row in ratio of 25 : 23. Ratio of eyes AME : ALE : PME : PLE = 6 : 3.5 (long diameter) : 12 : 12. AME separated from one another by eight sixths of their diameter, from ALE by fifty sixths of their diameter. PME separated from one another by seventeen thirteenths of their diameter, from PLE by thirty-four thirteenths of their diameter. Laterals separated by eight twelfths of diameter of PLE. Central ocular quadrangle wider behind than in front in ratio of 40 : 17, slightly longer than wide behind. AME slightly raised and directed somewhat laterally; LE on more conspicuously raised confluent tubercles; ALE free beneath.

ALE pearly white and somewhat irregularly oval; all others circular and dark. Width of clypeus equal to nine sixths of diameter of AME.

*Chelicerae.* — Not produced, parallel, rather more robust than usual in this genus, quite gibbous in front; basal segment 2.21 mm. long; well-developed boss; long yellowish hair in front. Fang fairly robust, evenly curved, finely dentelated along inner margin. Promargin of fang groove with three fairly strong teeth, middle one largest; retromargin with three larger teeth. Well-developed scopula only on promargin.

*Maxillae.* — Parallel, longer than wide in middle in ratio of 38 : 17, quite concave on outer margins, not dilated apically. Palp inserted into second fourth. Marginal serrula extending over distal lateral half. Well-developed scopula along distal medial half of anterior border and medial angle.

*Lip.* — Longer than wide in ratio of 25 : 15. Basal excavations extend slightly more than half of length of organ, which reaches two thirds of length of maxillae. Nearly rectangular. Distal border straight, with row of stiff converging black bristles. Sternal suture straight.

*Sternum.* — Longer than wide in ratio of 50 : 36, widest opposite posterior border of second coxae, covered with fine upright hair and scattered long slender bristles. Anterior end not much narrowed. Posterior end terminates in sharp point not extended between posterior coxae, which are separated by about one fourth of their diameter.

*Legs.* — 1243. Tibial index of first leg 12, of fourth leg 13.

| | *Femora* | *Patellae and tibiae* | *Metatarsi* | *Tarsi* | *Totals* |
|---|---|---|---|---|---|
| | (All measurements in millimeters) | | | | |
| 1. | 6.21 | 7.96 | 4.78 | 1.78 | 20.73 |
| 2. | 5.76 | 6.74 | 4.41 | 1.80 | 18.71 |
| 3. | 4.29 | 4.53 | 3.55 | 1.72 | 14.09 |
| 4. | 6.00 | 5.88 | 4.41 | 1.76 | 18.05 |

Ventral spines of first and second tibiae 2 – 2 – 2 – 2 – 0; of third tibiae 1p – 1p – 0; of fourth tibiae 1p – 2 – 0. Ventral spines of first and second metatarsi 2 – 2 – 2 – 0 (lateral spines easily confused with ventral spines); of third metatarsi 2 – 1p – 2; of fourth metatarsi 2 – 2 (?) – 1 (?). Ventral spines of first and second legs very

long and robust. Tibia and tarsus of palp very spiny. Palpal claw with seven teeth increasing in length from basal tooth. Trichobothria apparently distributed essentially as in *S. silvaticus*, sp. nov. Three claws throughout; two lateral claws each with about six or seven simple teeth in a single row. All legs with moderately well developed coat of short brown hair and scattered long yellowish hair. Brushes of hair frequently occurring ventrally on third and fourth tibiae and metatarsi in this genus apparently much reduced on third leg and subdivided on fourth tibiae into three ventral tufts.

*Abdomen.* — Longer than wide in ratio of 77 : 30, widest in the middle; mildly flattened. Anal tubercle flattened, with broad base. Spinnerets normal to genus. Soft, weakly developed colulus. Tracheal spiracle just anterior to colulus. At some distance in front of tracheal spiracle is transverse fold, significance of which I do not know. Good coating of short brownish hair; numerous short slender brown spines. Surface more or less pitted and grooved.

*Epigynum.* — Characterized by two chitinized hooklike lobes, which are slender, curved away from one another, and not intricately sculptured (Fig. 12).

*Color in alcohol.* — Essentially as described for *S. robustus*, sp. nov. Chelicerae deeper brown and terminal segments of spinnerets reddish brown.

*Type locality.* — Female holotype from Barro Colorado Island, Canal Zone, July, 1936. Female paratype from same locality, June, 1934.

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# AGE CHANGES IN THE TEETH OF THE COTTONTAIL RABBIT, *SYLVILAGUS FLORIDANUS*

LEE R. DICE AND DORA S. DICE

THE following descriptions of the teeth of the cottontail rabbit are based mostly on specimens of *Sylvilagus floridanus mearnsii* Allen. A few specimens of the related subspecies *alacer* and *similis* have, however, also been examined. All the individuals mentioned are in the collection of the Division of Mammals, Museum of Zoölogy, University of Michigan. The drawings were made by Grace Eager.

At birth the tips of the principal incisors ($I_1$, $I^1$) in both jaws of the young cottontail are already present, but none of the other teeth have cut the gums. The molariform teeth in both jaws show prominently under the gums at this time, however, and are evidently nearly ready to erupt. We base these statements on the condition of a young *alacer* from Rago, Kansas, which died in captivity immediately after birth and which is now preserved in alcohol (No. 58279).

The incisors which are present at birth are the permanent incisors, for in no specimens of older ages is there any indication of a replacement of these teeth. On the other hand, the second pair of upper incisors are preceded by milk teeth.

The next youngest skull available to us (No. 77823) is from a young *mearnsii* taken from a nest near Ann Arbor, Michigan. In the lower jaw the incisors ($I_1$) show hardly any wear. The two deciduous molars ($DM_3$, $DM_4$) apparently had already cut through the gum, but are unworn. The first molar ($M_1$) also is well in place, and may likewise have pierced the gum. The second molar ($M_2$) is below the level of the alveolus. The third molar is not present, probably having been lost in the preparation of the specimen. In the upper jaw the anterior incisor ($I^1$) already shows a considerable amount of wear. The posterior deciduous incisor ($DI^2$) is present and probably was above the gum. The tips of the three deciduous

molars ($DM^2$, $DM^3$, $DM^4$) and of the first molar ($M^1$) had probably also cut the gum. The second molar ($M^2$) is present, but is barely above the alveolus. The third molar ($M^3$) is not in sight.

Another individual (No. 77829) from the same nest as the animal described in the preceding paragraph was kept in captivity for 10 days before being prepared as a specimen. The teeth reveal a considerable advance over those of the younger animal, but the artificial feeding which this rabbit received may have delayed somewhat the usual rate of growth. The lower incisors ($I_1$) already exhibit a slight amount of wear. The first three molariform teeth ($DM_3$, $DM_4$, $M_1$) show traces of wear on the cusps, and the third premolar ($P_3$) appears beneath its milk molar. The second molar ($M_2$) is barely above the alveolus, and is not yet erupted. The third molar is not present. In the upper jaw there has been a considerable amount of wear on the front incisor ($I^1$). The deciduous posterior incisor ($DI^2$) is present, and just behind it appears the tip of the permanent second incisor ($I^2$), which is still within its alveolus. The first four molariform teeth ($DM^2$, $DM^3$, $P^4$, $M^1$) all have traces of wear on their cusps. The third and fourth permanent premolars ($P^3$, $P^4$) are present in the alveoli below their deciduous predecessors; $M^2$ is not yet erupted; and $M^3$ is missing.

Another series is available of three skulls of young animals captured in the nest near Ann Arbor, kept as captives, and prepared as specimens one week apart. The youngest (♀ No. 75496), killed when her eyes were not yet open, was of approximately the same age as No. 77823, described above, and the teeth are in similar condition. The next older specimen (♀ No. 75497), which had the eyes open, is slightly larger than specimen No. 77829, described above, and the teeth are more worn. The oldest specimen (♂ No. 75498) was two weeks older when killed than the youngest individual, and differs in the much larger teeth. This animal was fully haired and had a total length of 142 mm., and was probably nearly ready to leave the nest. The lower incisor is well worn. The two lower deciduous molars ($DM_3$, $DM_4$) are also well worn and are about to be displaced by permanent premolars. The first two lower molars ($M_1$, $M_2$) exhibit some wear. The last molar ($M_3$) is present in the alveolus, but has not yet erupted. The anterior upper incisor ($I^1$) is well worn and has a prominent internal pit. The posterior deciduous incisor ($DI^2$) is still present, and the tip of the permanent posterior

incisor ($I^2$) is probably just erupting. The upper deciduous molars ($DM^2$, $DM^3$, $DM^4$) are much worn and are about to be replaced by the permanent premolars. The first two molars ($M^1$, $M^2$) show a small amount of wear. $M^3$ is deep in its alveolus, not yet ready to erupt.

In a specimen (No. 52708) with a total length of 170 millimeters, from Three Oaks, Michigan, all the deciduous molars are still in place. The deciduous posterior upper incisor ($DI^1$) is not yet lost, and the permanent posterior incisor ($I^2$) is still below the gum.

Two young specimens from Alma, Michigan, taken on the same day, have total lengths of 208 and 193 millimeters, and may have come from the same brood. In the smaller specimen both of the much-worn upper deciduous incisors ($DI^2$) are present; in the larger specimen the left one has been lost. The permanent posterior incisors are in place, and their tips have probably erupted. In the smaller specimen all the deciduous molars are present, though much worn. In the larger specimen all the deciduous molars are present except the two on the right lower jaw. The two right deciduous molars have obviously just been lost, for the two premolars are entirely unworn. The lower and upper third molars in both specimens have risen above their alveoli, but have probably not yet cut the gums.

The next older specimen available is a female from Portage Lake, Washtenaw County, Michigan (No. 53757). The animal was not measured, but it weighed 273 grams. The upper and lower third molars are both in place and show evident wear. All the deciduous teeth are gone. A male (No. 57861) with a skull of nearly the same size, from Ladysmith, Wisconsin, also has all the permanent teeth in place. This animal had a total length of 267 millimeters.

It is evident, then, that the permanent set of teeth is complete in these cottontails when the young animals have reached a total length of about 250 millimeters and weigh about 250 grams. At this time they are probably a little more than one month of age.

The patterns exhibited on the faces of the teeth by the layers of dentine, enamel, and cement change somewhat with the age of the animal. In describing these patterns we are unable to give an account of the condition of very old, worn-out teeth, because no extremely old specimen has been available to us.

There is a small amount of individual variation in the details of

enamel folding on the borders of the reëntrant angles of the molariform teeth. Except, however, for the slight changes correlated with advance in age which we here describe and except for the occasional doubling of the anterior reëntrant angle of $P_3$, the main features of enamel pattern on the teeth are much alike in all the specimens of this species which we have examined.

The upper first incisors ($I^1$) are provided with a prominent anterior groove (Fig. 1), which is present on the tooth at the birth of the animal and persists throughout life. An interior pit of enamel occurs in very young animals, but quickly disappears. The anterior face of the incisor has a heavy border of enamel, but enamel is lacking on the posterior part of the tooth. The result of wear is to give the incisor its characteristic chisel shape.

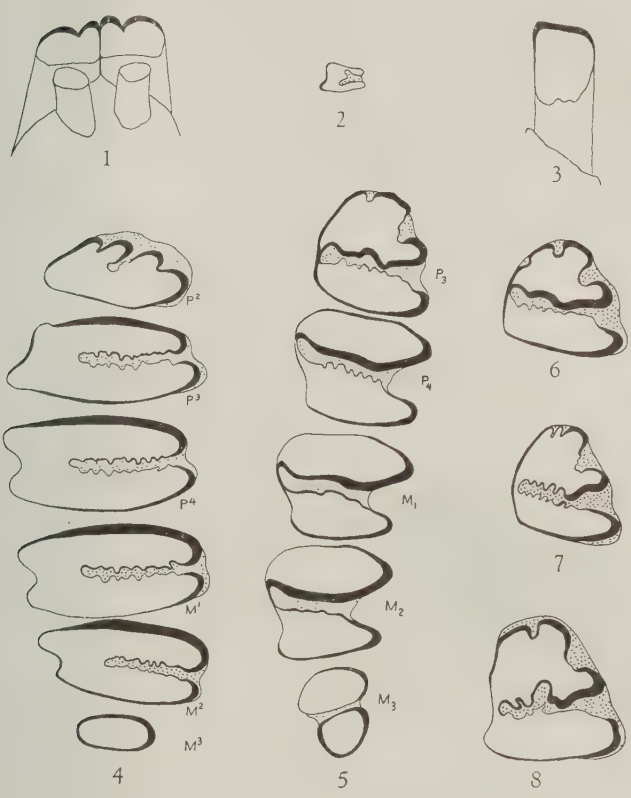
The deciduous upper second incisor ($DI^2$) is only a small peg, which is soon lost. The permanent second incisor ($I^2$) also is peglike (Fig. 1) and is completely surrounded by a thin band of enamel. This tooth is usually worn off nearly flat. It probably serves as a stop to prevent the sharp lower incisor from injuring the palate.

The most anterior upper deciduous molar ($DM^2$) is an oval tooth which has a strong anterior reëntrant angle. The angle is especially strong on the crown of the tooth, which it divides unequally into two cusps (Pl. I). The labial cusp is larger and stronger than the lingual one. Two shallow grooves, one on each side of the main anterior reëntrant angle, occur on the lower anterior face of the tooth. These grooves do not appear in our plate.

The upper third milk molar ($DM^3$) in all the specimens available to us shows a small amount of wear on the face of the tooth (Pl. I), and the characters of its cusps when first erupted cannot, therefore, be determined. The tooth, when slightly worn, has a strong lingual reëntrant angle, which bifurcates at its internal end to form an enamel Y (Fig. 2). This Y is assumed to represent a trace of an enamel crescent like those which occur on the molariform teeth of many primitive lagomorphs. The enamel of the Y seems to be at no time connected with the enamel of the exterior of the tooth, except through the reëntrant angle.

The upper fourth deciduous molar ($DM^4$) is similar to $DM^3$ in all essential characters, including an enamel Y on its face.

The anterior upper premolar ($P^2$) in the mature animal has three anterior reëntrant angles, each of which slopes toward the labial




ENAMEL PATTERNS ON FACES OF TEETH OF
*SYLVILAGUS FLORIDANUS MEARNSII*

1. Upper incisors ($I^1$ and $I^2$). No. 57109. × 6
2. Right upper third deciduous molar ($DM^3$). No. 75497. × 4
3. Right lower incisor ($I_1$). No. 57109. × 4
4. Right upper molariform teeth. No. 55174. × 6
5. Right lower molariform teeth. No. 55174. × 6
6. Right $P_3$, showing unusual reëntrant angle on the anterior lingual side of the tooth. No. 58305. × 6
7. Right $P_3$ from moderately old animal. No. 54206. × 6
8. Right $P_3$ from very old animal. No. 42182. × 6

side of the tooth (Fig. 4). In the newly erupted tooth (No. 53757) only the middle one of these angles has its full depth. A slight amount of wear soon discloses the two lateral angles, which, however, are only about half the depth of the middle angle. All these angles persist and become somewhat more prominent in the most aged specimens at hand. A thick rim of enamel completely surrounds this tooth. In the mature animal a layer of cement covers the anterior and lingual surfaces of the tooth and fills the anterior reëntrant angles.

The upper third premolar ($P^3$), when first uncovered by the removal of its milk molar, exhibits on its face an enamel crescent connecting with the inner end of the lingual reëntrant angle (Pl. I). The crescent is quickly removed by wear, and the tooth then shows the lingual reëntrant angle as its most prominent character. In the young animal the enamel on both borders of this reëntrant angle is slightly wavy. In older animals the enamel becomes more strongly crenulated (Fig. 4). At all ages the enamel of the anterior border of the angle tends to be slightly heavier and sometimes more crenulated than that of the posterior border. The enamel on the outside of the tooth is heaviest on the lingual surface and diminishes in thickness toward the labial side. Enamel is entirely lacking on the labial surface. Cement fills the lingual reëntrant angle and also covers the lingual surface of the tooth.

The upper fourth premolar ($P^4$) when first uncovered shows on its face traces of an enamel crescent like that on $P^3$. The later stages of wear on this tooth also are similar to those of $P^3$.

The upper first and second molars ($M^1$ and $M^2$) when first erupted appear to be two separate pillars (Pl. I), but they are joined just beneath the top of the tooth, and the connection becomes evident as soon as wear begins on the crown (Fig. 4). Parts of an enamel crescent appear fleetingly on the faces of each of these teeth. The two molars in the adult have enamel patterns very similar to those of the adjacent premolars ($P^3$ and $P^4$).

The upper third molar ($M^3$) is a peglike tooth at its first appearance, but with wear its face assumes an elongated form, somewhat irregular in outline (Fig. 4). Enamel completely surrounds this tooth.

The permanent lower incisor ($I_1$) is present at birth (No. 77823), at which time it has a prominent anterior groove. This groove quickly disappears with wear, and subsequently the tooth has the

typical chisel shape, with the anterior edge nearly straight across (Fig. 3). A heavy band of enamel covers the anterior face of the incisor, but does not extend over the posterior part.

The anterior lower deciduous molar ($DM_3$) when first erupted consists of two enamel pillars, on the anterior one of which there is a prominent anterior lobe (Pl. I). With wear the anterior lobe becomes merged with the anterior pillar, and is represented only by a strong labial reëntrant angle. At the latest stages of wear the two enamel pillars remain unconnected.

The posterior lower deciduous molar ($DM_4$) when first erupted also consists of two independent enamel pillars (Pl. I). With wear they become connected along the lingual edge of the tooth by a rim of enamel, and at this time the tooth has the same general pattern as the permanent premolar ($P_4$), which soon replaces it.

The anterior lower premolar ($P_3$), when first exposed by the removal of its milk molar, consists of two closely fused pillars (Pl. I). The anterior pillar rises a little higher than the posterior one. There is no obvious enamel pattern on the face of the tooth in our specimen (No. 62765), but only some irregular markings, which may be due in part to wear. There is no posterior lobe on this tooth, such as that which is present for a time on $P_4$.

At its first stage of wear $P_3$ is made up of two separate enamel ovals connected on the lingual edge of the tooth by a thin line of enamel (No. 53757). With continued wear the line of enamel joining the two ovals becomes doubled (No. 57109), and the tooth is then composed of two closely approximated pillars separated by a labial reëntrant angle, which completely crosses the tooth (Fig. 5). In one specimen an unusual shallow groove is present on the anterior lingual border of the tooth (Fig. 6). In old animals the main reëntrant angle becomes somewhat shorter and then fails completely to cross the tooth (Figs. 7–8). In the oldest animals available to us as specimens, however, the main reëntrant angle of $P_3$ does not lack very much of crossing the tooth, and it therefore never approaches the character of *Hypolagus*, in which genus the reëntrant angle extends only about halfway across.

An obtuse, posteriorly directed cape or point on the anterior lip of the main reëntrant angle of $P_3$ is a characteristic feature of *Sylvilagus floridanus* (Fig. 5). It is especially prominent in young animals. The portion of the anterior lip of the reëntrant angle internal to the

cape is smooth or slightly wavy in young rabbits (Fig. 5), tends to become crenulated in middle age (Fig. 7), and is often rather coarsely folded in old animals (Fig. 8). The enamel is much thicker on the anterior lip of the main reëntrant angle than on the posterior lip.

A widely open reëntrant angle occurs on the labial side of $P_3$ just anterior to the main reëntrant angle (Fig. 5). It extends about one fourth the way across the tooth. It appears at the first stage of wear and is present in the oldest specimens we have examined. The enamel making up this reëntrant angle is occasionally somewhat folded.

A shallow reëntrant angle appears on the anterior face of $P_3$ at the first stage of wear (Pl. I). With continued wear this anterior angle becomes deeper and narrower (Fig. 4). In rare specimens two anterior reëntrant angles occur side by side (Fig. 7).

Cement fills the reëntrant angles of $P_3$ and covers the anterior border of the tooth in a thin layer.

The lower fourth premolar ($P_4$) when it first appears consists of two pillars (Pl. I). There is a small lobe on the posterior border of the tooth, but it disappears as soon as wear begins. The two enamel pillars making up $P_4$ are at first connected only by cement (No. 31593). With a small amount of wear the two pillars become connected at the lingual side of the tooth by what appears to be a single line of enamel (No. 62765). In older specimens, however, the connecting ridge of enamel between the two pillars is obviously double (Fig. 5). The enamel around the anterior pillar is heavier than that around the posterior pillar, and therefore the posterior pillar wears down more rapidly than the anterior one. The anterior pillar, accordingly, stands higher than the posterior pillar at all stages of wear. The enamel on the anterior border of the posterior pillar (posterior lip of the reëntrant angle) is very thin and in young animals is usually slightly wavy, but in older animals it is crenulated (Fig. 5). Cement fills the reëntrant angle.

The first two lower molars ($M_1$, $M_2$) when first erupted each consist of two independent pillars (Pl. I). On the face of $M_1$ before it has cut the gum there may be seen on some specimens (No. 77823) a trace of the posterior cusp mentioned above as fleetingly present on $P_4$. In the early stages of wear the two pillars making up each of these molars become connected on the lingual side of the tooth by a thin line of enamel, and in later stages of wear this is double (Fig. 5).

The history of enamel wear on these molars therefore closely follows that on $P_4$.

The last lower molar ($M_3$) consists at all stages of wear of two independent pillars, of which the posterior one is the smaller (Fig. 5). Cement fills the space between the two pillars.

In the oldest specimens available to us there is no indication that any of the permanent molariform teeth are approaching the limit of their possible growth. All are fully hypsodont, and there is no suggestion in any of them of the formation of a root, with consequent cessation of growth.

## DISCUSSION

After the young cottontail has acquired its full set of permanent teeth, probably at about one month of age, the teeth no longer offer much assistance in determining its age. In old animals the main reëntrant angle on $P_3$ fails completely to cross the tooth, and there is some increase in heaviness of enamel and in degree of crenulation on the internal reëntrant angles, but the differences between these characters in old and young animals are rather slight. It is possible, however, that in animals older than any we have examined the enamel patterns may show some degeneration.

The Y-shaped peninsulas of enamel which occur at an early stage of wear on the faces of $DM^3$ and $DM^4$ and the enamel crescents which are fleetingly present on the unworn crowns of $P^3$ and $P^4$ of *Sylvilagus floridanus* resemble a type of enamel pattern which was much more important in the early lagomorphs. Enamel crescents were conspicuous on many of the upper molariform teeth of the Eocene *Mytonolagus petersoni* (Burke, 1934, Pl. L); they persisted over a considerable period of wear on the Oligocene *Palaeolagus haydeni* (Troxell, 1921, Figs. 9–16); and they were perhaps of lesser importance on the Upper Oligocene *Archaeolagus ennisianus* (Dice, 1917, Fig. 1). These crescents are very prominent on some of the upper teeth of the fossil and Recent ochotonids. Crescents occur also for a brief time on some of the newly erupted upper molariform teeth of the Recent leporids *Oryctolagus cuniculus*, *Lepus timidus*, and *Sylvilagus brasiliensis* (Major, 1899, Pl. 36). We therefore interpret the enamel crescents of *S. floridanus* as fleeting vestiges of an ancestral character.

The small posterior lobe which is present briefly on the newly uncovered crowns of $P_4$ and $M_1$ of *Sylvilagus floridanus* seems also to be a vestige of a character which is important in the primitive

rabbits. Posterior lobes which persisted during a considerable period of wear were present on many of the lower molariform teeth of the Oligocene *Palaeolagus haydeni* (Dice and Dice, 1935, Figs. 8–10, 14, 15). Posterior lobes are present, however, in our modern cottontail, so far as we are able to discover, only on $P_4$ and $M_1$, and they promptly disappear when wear begins on the teeth.

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## EXPLANATION OF PLATE I

Molariform teeth of *Sylvilagus floridanus mearnsii*

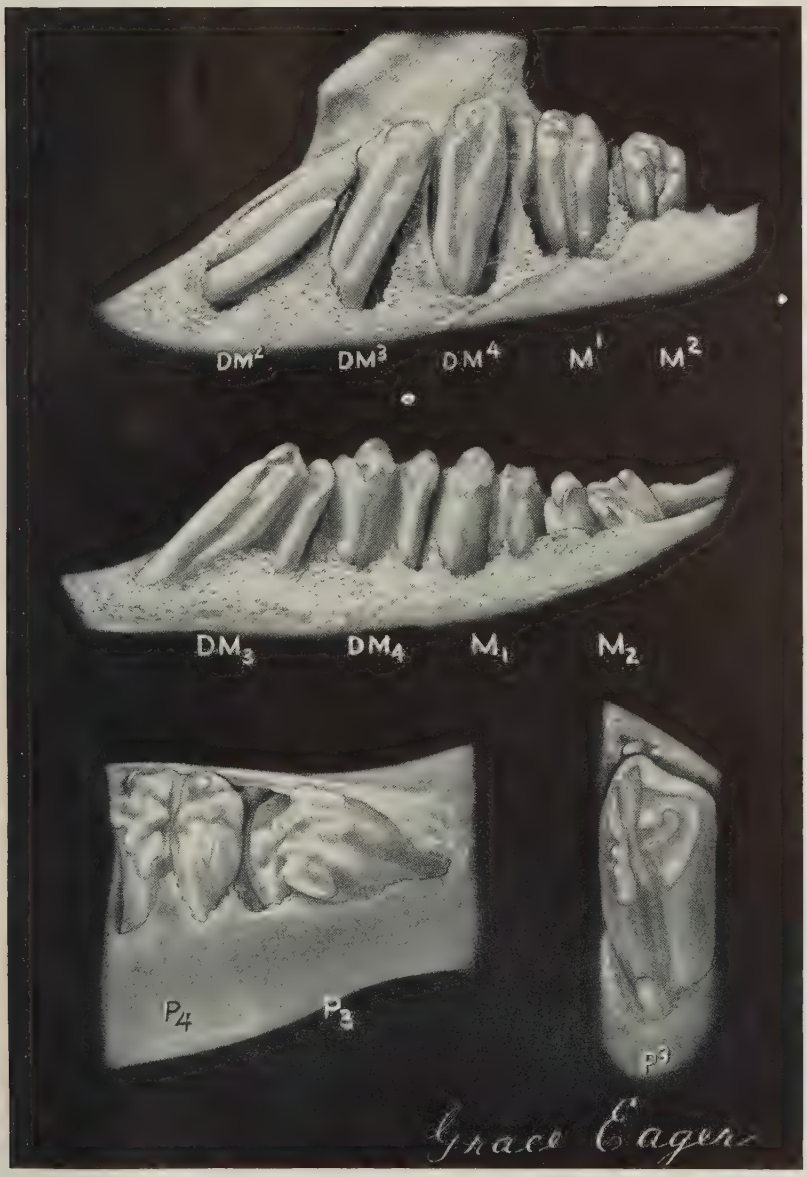
Magnified about 12 times.

Upper figure. Left upper molariform teeth, showing early stages of wear on the deciduous molars. The first two permanent molars are just erupting. No. 75496

Middle figure. Left lower molariform teeth of the same individual

Lower left figure. Newly uncovered right lower premolars ($P_3$ and $P_4$). No. 58953

Lower right figure. Early stage of wear on the right upper third premolar ($P^3$), showing trace of enamel crescent. No. 37574




*Sylvilagus floridanus mearnsii*

# INCREASED NUMBER AND DELAYED DEVELOPMENT OF SCALES IN ABNORMAL SUCKERS

CARL L. HUBBS

THE evidence presented in this paper is a new confirmation of the thesis that the countable and measurable characters of fishes are to a large degree determined or modified by the rate of development (Hubbs, 1926a).

In preparing a report on their survey of the fishes of the Connecticut River watershed in New Hampshire Joseph R. Bailey and James A. Oliver (1940, p. 180, fig. 80) discovered a number of teratological suckers which were referred to me for identification. The variant characters seemed of sufficient interest and significance to warrant an analytical study, for which the collectors obligingly made the material available.

The abnormal suckers were collected on August 25, 1939, in Nash Bog Pond and its outlet, Nash Stream, a tributary of the Upper Ammonoosuc River of the Connecticut River system in New Hampshire. Some of the abnormal fish came from the artificial mud-bottom pond, but most of them were taken in the outlet just below the dam. The rather clear light-brown water had a temperature of 70° F. in midafternoon, when the reading for the air was the same. The current in the outlet was moderate. The bottom of rubble, boulders, silt, and muck supported a sparse and scattered growth of vegetation. Further ecological data are given on pages 21, 69, 91, and 110 of the Survey Report containing the paper by Bailey and Oliver (1940).

There is nothing particularly unusual in the local conditions that might be responsible for the abnormal characters of the suckers. Nor did an examination of the fish disclose a possible cause of the aberrancy. They do not appear emaciated; on the contrary, they are thick, deep, and pudgy, as though very well fed. As can be seen from Table IV, the abnormal specimens average larger than

## TABLE I

LATERAL-LINE SCALE COUNTS IN SUCKERS FROM THE CONNECTICUT RIVER SYSTEM IN NEW HAMPSHIRE

| Lateral-line scales | *Catostomus commersonnii commersonnii* | | | *Catostomus catostomus nannomyzon* | |
|---|---|---|---|---|---|
| | Other localities | Nash Bog Pond | | Nash Bog Pond | Other localities* |
| | | Normal | Abnormal | | |
| 57 | 2 | .. | .. | .. | .. |
| 58 | 3 | 4 | .. | .. | .. |
| 59 | 7 | 1 | .. | .. | .. |
| 60 | 4 | 3 | .. | .. | .. |
| 61 | 5 | 8 | .. | .. | .. |
| 62 | 2 | 12 | .. | .. | .. |
| 63 | 3 | 6 | .. | .. | .. |
| 64 | .. | 3 | .. | .. | .. |
| 65 | 1 | 2 | .. | .. | .. |
| 66 | 1 | 2 | .. | .. | .. |
| 67 | .. | 2 | .. | .. | .. |
| 68 | .. | .. | .. | .. | .. |
| 69 | .. | .. | .. | .. | .. |
| 70 | .. | .. | .. | .. | .. |
| 71 | .. | .. | .. | .. | .. |
| 72 | .. | .. | .. | .. | .. |
| 73 | .. | .. | .. | .. | .. |
| 74 | .. | .. | .. | .. | .. |
| 75 | .. | .. | .. | .. | .. |
| 76 | .. | .. | .. | .. | .. |
| 77 | .. | .. | .. | .. | .. |
| 78 | .. | .. | .. | .. | .. |
| 79 | .. | .. | .. | .. | .. |
| 80 | .. | .. | .. | .. | .. |
| 81 | .. | .. | .. | .. | 2 |
| 82 | .. | .. | .. | .. | .. |
| 83 | .. | .. | .. | .. | .. |
| 84 | .. | .. | .. | .. | .. |
| 85 | .. | .. | .. | .. | .. |
| 86 | .. | .. | .. | .. | 4 |
| 87 | .. | .. | .. | .. | 3 |
| 88 | .. | .. | 1 | .. | 3 |
| 89 | .. | .. | .. | .. | 1 |
| 90 | .. | .. | 2 | .. | 6 |
| 91 | .. | .. | .. | .. | 5 |
| 92 | .. | .. | 1 | .. | 9 |
| 93 | .. | .. | .. | .. | 2 |
| 94 | .. | .. | .. | .. | 4 |
| 95 | .. | .. | .. | .. | 3 |
| 96 | .. | .. | 1 | .. | 1 |
| 97 | .. | .. | 1 | .. | 3 |
| 98 | .. | .. | .. | 1 | 1 |
| 99 | .. | .. | .. | 1 | 2 |
| 100 | .. | .. | .. | 1 | 1 |
| 101 | .. | .. | 1 | .. | 1 |
| Number | 28 | 43 | 7 | 3 | 51 |
| Average | 60.36 | 62.07 | 93.43 | 99.00 | 91.65 |

* Counts from Bailey and Oliver (1940), less one count of 99 scales for a specimen from Nash Bog Pond.

the unmodified individuals of the same species in the same collection. The guts, like those of the normal examples, are filled with cladocerans, a very common food of young suckers (Bigelow, 1924). Since almost precisely parallel abnormalities have been attributed to a helminth infestation (Hubbs, 1927), a search was made for internal parasites in two of the terata, but they were found relatively free of infestations. The evidence therefore negates the suggestion of Bailey and Oliver (1940, p. 180) that the abnormalities were due to

TABLE II

Dorsal-Ray Counts in Suckers from the Connecticut River System in New Hampshire

| | Principal dorsal rays | | | | | | |
|---|---|---|---|---|---|---|---|
| | 9 | 10 | 11 | 12 | 13 | No. | Av. |
| *Catostomus commersonnii commersonnii* | | | | | | | |
| Nash Bog Pond | | | | | | | |
| Abnormal individuals, 21 to 37, mostly 24 to 35 mm. | .. | .. | 8 | 25 | 1 | 34 | 11.79 |
| Normal individuals: | | | | | | | |
| 16 to 24 mm. | .. | 7 | 81 | 57 | 3 | 148 | 11.38 |
| 25 to 43 mm. | .. | .. | 20 | 33 | 2 | 55 | 11.66 |
| Other localities | .. | .. | 3 | 23 | 4 | 30 | 12.03 |
| *Catostomus catostomus nannomyzon* | | | | | | | |
| Nash Bog Pond | .. | 2 | 1 | .. | .. | 3 | 10.33? |
| Other localities | 6 | 104 | 3 | .. | .. | 113 | 9.97 |

parasitism or a silt diet. A genetic basis is suspected, but an environmental cause is by no means ruled out.

When the abnormal suckers were identified as *Catostomus commersonnii commersonnii,* their most striking feature (shown, however, only by the larger individuals) was an almost unbelievable increase in the number of scales (Table I). Instead of ranging from 58 to 67 (an expected range), as in the unmodified specimens of the species from this locality, the scales of the variants numbered from about 88 to 101. The counts of 96, 97, and 101 were approximately certain, whereas those of 88 to 92 were less sure (but may be taken as minimum enumerations). In the other abnormal individuals the squamation was not sufficiently developed to allow an accurate count, but in all specimens which were partially scaled posteriorly (see Table III)

the scales were of the same order of minuteness as in the specimens that were counted.

Why, then, are these abnormal suckers not referred to the fine-scaled form, *Catostomus catostomus nannomyzon*, of which three

## TABLE III

DEVELOPMENT OF THE SQUAMATION IN SUCKERS FROM THE CONNECTICUT RIVER SYSTEM IN NEW HAMPSHIRE

| Standard length in mm. | 0 | | | 1 | 2 | | | 3 | 4 | 5 | | 6 | | 7 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | (A) | C | (A) | N | (A) | C | N | N | N | C | N | C | N | C |
| 14 | 3 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 15 | 5 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 16 | 22 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 17 | 21 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 18 | 36 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 19 | 36 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 20 | 21 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 21 | 7 | (1) | 1 | .. | 3 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 22 | .. | (1) | .. | .. | 12 | .. | 1 | .. | .. | .. | .. | .. | .. | .. | .. |
| 23 | .. | (1) | .. | .. | 4 | .. | .. | 3 | .. | .. | .. | .. | .. | .. | .. |
| 24 | .. | (4) | .. | .. | 3 | .. | .. | 5 | .. | .. | .. | .. | .. | .. | .. |
| 25 | .. | (5) | .. | .. | .. | .. | .. | 3 | 3 | .. | .. | .. | .. | .. | .. |
| 26 | .. | (7) | .. | .. | .. | .. | .. | 1 | 3 | 2 | .. | .. | .. | .. | .. |
| 27 | .. | (2) | .. | .. | .. | .. | .. | .. | 2 | 6 | .. | .. | .. | .. | .. |
| 28 | .. | (2) | .. | .. | .. | .. | .. | .. | .. | 6 | .. | .. | .. | .. | .. |
| 29 | .. | (2) | .. | .. | .. | .. | .. | .. | .. | 8 | 1 | .. | .. | .. | .. |
| 30 | .. | .. | .. | (1) | .. | (1) | .. | .. | .. | 5 | .. | .. | .. | .. | .. |
| 31 | .. | .. | .. | (6) | .. | (2) | .. | .. | .. | 4 | 1 | .. | .. | .. | .. |
| 32 | .. | .. | .. | (1) | .. | (4) | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 33 | .. | .. | .. | .. | .. | (1) | .. | .. | .. | .. | 1 | .. | .. | .. | .. |
| 34 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 2 | 1 | .. | .. | .. |
| 35 | .. | .. | .. | .. | .. | (3) | .. | .. | .. | .. | 2 | .. | .. | .. | 1 |
| 36 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 1 | 2 | .. | .. | .. |
| 37 | .. | .. | .. | .. | .. | (1) | .. | .. | .. | .. | .. | 3 | .. | .. | .. |
| 38 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 1 | .. | 1 | 1 |
| 39 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 40 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 1 | .. | .. | 2 | 1 |
| 41 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 42 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 1 | .. | .. |
| 43 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 1 | 3 |
| 44 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 3 |
| 45 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 3 |
| 46 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 3 |
| 47 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 4 |
| 48 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 3 |
| 49 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 2 |

specimens were collected at the same time and place? The agreement in the size of the scales is close. Other characters, however, point definitely to the identification of the variant fish as *C. commersonnii commersonnii*. As in the latter form, the short, blunt, and scarcely protruding snout is decidedly less elongate and prominent than in even the very small young of *C. catostomus*, recently transformed from the postlarval stage. The juvenile color pattern (little vermiculated, with three conspicuous dark blotches) is more like that of *commersonnii* than that of *catostomus*.

Furthermore, the dorsal rays (Table II) are typical of *Catostomus commersonnii commersonnii* and differ from those of *C. catostomus nannomyzon*. The average approximates that for the specimens of *C. commersonnii* from other localities in the Connecticut River system

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EXPLANATION OF SYMBOLS AND DATA IN TABLE III

The degree of completeness of the squamation is given in eight columns by the numbers 0 to 7, indicating stages of development as follows:

0 — No scales evident even on caudal peduncle.
1 — Scales evident only on mid-sides of tail region.
2 — Evident scales confined to a median strip, broadest toward caudal, but also developed just behind shoulder girdle.
3 — Squamation complete except on back before caudal peduncle.
4 — Squamation nearly complete except on back above mid-sides and before middle of dorsal fin.
5 — Squamation complete except over most of nape.
6 — Squamation complete except down middle of nape.
7 — Squamation complete.

No account was taken of the squamation of the lower parts anteriorly, and the scales were interpreted as present or absent by a surface examination with a high-powered binocular microscope, under a bright light.

The columns have from one to three sets of figures. These figures represent the number of specimens, of the given length, which were interpreted as being in the stage of development indicated at the head of the columns, as follows:

N — *Catostomus commersonnii commersonnii* from Nash Bog Pond. Normal specimens.

(A) — *Catostomus commersonnii commersonnii* from Nash Bog Pond. Abnormal specimens with retarded development (numbers in parentheses).

C — *Catostomus catostomus nannomyzon* from various localities in the Connecticut River system of New Hampshire. Several other young, more than 50 mm. long, were all in stage 7. The two young from Nash Bog Pond measured 40 mm. (stage 5) and 49 mm. (stage 7).

The size of the normal specimens of *Catostomus commersonnii* at the time of initial scale formation corresponds with the figure of "somewhat less than 22 millimeters," stated by Stewart (1926, p. 169).

in New Hampshire, and is somewhat higher than that for the normal specimens in the same collection. The agreement is close, however, when the abnormal specimens are compared with normal ones of like size (as indicated in Table II). The lower average number of dorsal rays in the smaller unmodified suckers of the August 25 collection may be another example of seasonal variation, by which the fish hatched in the warmer water show the fewer segments (see Hubbs, 1922, 1923, 1925, 1926a, 1934). That all fish listed in Table II represent young of the year seems obvious from the contributions of Bigelow (1924) and Hubbs and Creaser (1924).

The teratological suckers (Pl. I) were picked out by reason of the retention of larval features, indicative of abnormally delayed differentiation. The whole texture is soft and weak. Since the mouth is exceptionally oblique, as normally in very much smaller suckers (Stewart, 1926), the posterior angle of the mandible is much more conspicuous than usual. The body wall over the coelom is very flabby and transparent, largely because the superficial muscles are weakly developed; the heart and the ribs are clearly evident in external view. The pectoral fins retain a distinct trace of the larval pedunculation. The development of the squamation has been greatly retarded (Table III), as is further emphasized below.

The correctness of the separation of the abnormal specimens on the basis of these characters was fully confirmed when it was observed that the gill membranes in all the aberrant examples had remained free from the isthmus a strikingly longer time than in normal development (Table IV). This seems to be an unusual type of retention of a larval character, which I recall having otherwise seen only in a collection of *Margariscus margarita nachtriebi* from near the northern limit of its range (the specimens recorded by Cowan, 1939, p. 97); in this series most of the adults had the membranes free.

Postlarval habits as well as structures seem to have been retained by these teratological fish. Young suckers of their size (21 to 37 mm. in standard length) would be expected to have taken up a bottom habitat (Stewart, 1926, pp. 161 and 173, and personal observation). The collectors observed, however, that the abnormal fish were swimming about jerkily in short movements near the surface, therefore after the fashion of postlarval and very young juvenile suckers.

The important point in the present study is that some of the suckers from Nash Bog Pond in New Hampshire exhibit a greatly

TABLE IV

CHANGES, INDICATED BY FREQUENCIES, IN THE CHARACTER OF THE GILL MEMBRANES IN NORMAL AND IN ABNORMAL SUCKERS (*CATOSTOMUS COMMERSONNII COMMERSONNII*) FROM NASH BOG POND, NEW HAMPSHIRE

| Standard length in mm. | Gill membranes free from isthmus or nearly so | | Gill membranes partly attached to isthmus | | Gill membranes wholly attached to isthmus | |
|---|---|---|---|---|---|---|
| | Normal | Abnormal | Normal | Abnormal | Normal | Abnormal |
| 14 | 3 | .. | .. | .. | .. | .. |
| 15 | 5 | .. | .. | .. | .. | .. |
| 16 | 14 | .. | 8 | .. | .. | .. |
| 17 | 5 | .. | 16 | .. | .. | .. |
| 18 | .. | .. | 23 | .. | .. | .. |
| 19 | .. | .. | 17 | .. | 19 | .. |
| 20 | .. | .. | 3 | .. | 18 | .. |
| 21 | .. | 1 | .. | .. | 10 | .. |
| 22 | .. | 1 | .. | .. | 12 | .. |
| 23 | .. | 1 | .. | .. | 7 | .. |
| 24 | .. | 4 | .. | .. | 8 | .. |
| 25 | .. | 5 | .. | .. | 6 | .. |
| 26 | .. | 7 | .. | .. | 6 | .. |
| 27 | .. | 2 | .. | .. | 8 | .. |
| 28 | .. | 2 | .. | .. | 6 | .. |
| 29 | .. | 2 | .. | .. | 8 | .. |
| 30 | .. | 1 | .. | 1 | 5 | .. |
| 31 | .. | 3 | .. | 4 | 4 | 1 |
| 32 | .. | 2 | .. | 2 | .. | 1 |
| 33 | .. | .. | .. | .. | .. | 1 |
| 34 | .. | .. | .. | .. | 1 | .. |
| 35 | .. | 1 | .. | .. | .. | 2 |
| 36 | .. | .. | .. | .. | 2 | .. |
| 37 | .. | .. | .. | .. | 3 | 1 |
| 38 | .. | .. | .. | .. | 2 | .. |
| 39 | .. | .. | .. | .. | .. | .. |
| 40 | .. | .. | .. | .. | 2 | .. |
| 41 | .. | .. | .. | .. | .. | .. |
| 42 | .. | .. | .. | .. | .. | .. |
| 43 | .. | .. | .. | .. | 1 | .. |

delayed development of certain structures, including the scales (the reason seems relatively insignificant in the present interpretation), and that the scales show an increase of about 50 per cent in number. The explanation appears to be that the scale foci are laid down at approximately constant absolute distances from one another, whether the squamation develops early or late (Hubbs, 1927, pp. 93–94).

When scale formation is retarded while general growth is continued, there results a larger base on which the scales may develop. The number of scales which do form seems to be at least roughly proportionate to the area available.

In geographical as well as teratological variation there is considerable evidence that delayed lepidogenesis causes an increase in the number of scales. Thus in *Notropis cornutus* the final stages of scale formation, across the nape, are protracted toward the north, in correlation with the great increase northward in the number of predorsal scales (data in Hubbs, 1926b, pp. 38 and 46, and Hubbs and Brown, *in* Ortenburger and Hubbs, 1927, pp. 129–131).

It may now be regarded as reasonably well established that modifications in the rate of growth and of differentiation alter the characters of fishes, and play an important rôle in speciation.

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PLATE I

Teratological suckers from Nash Bog Pond, New Hampshire

The photograph, by F. W. Ouradnik, is used here by courtesy of the New Hampshire Fish and Game Department and Joseph R. Bailey

# LEUCOCYTIC REACTION TO BACTERIAL INFECTION IN ANIMALS*

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CERTAIN changes have been noted in the circulating polymorphonuclear leucocytes in men and the lower mammals during infections associated with the production of pus. Summarized briefly they are: (1) increased basophilia (acid reaction) of the cytoplasmic granules; (2) pycnosis of the nucleus (deep staining and clumping of the chromatin); (3) formation of basophilic nongranular areas (Döhle bodies) in the cytoplasm; and (4) vacuolization of the cytoplasm. When the bone marrow is unable to supply mature polymorphonuclear leucocytes in adequate numbers immature forms appear (the so-called "shift to the left").

In a series of experiments (O'Reilly, 1936) in which large doses of *Staphylococcus aureus* were injected into the rat an infectious state was produced and was accompanied by basophilia of the granules of the neutrophils, similar to that which is found in man during the course of a pyogenic infection.

To analyze the process of leucocytic behavior and immunity in man certain aspects of it have been studied in lower animals which are normally exposed to infecting media in their environment.

## MATERIAL AND METHODS

In this analysis *S. aureus* (from a case of osteomyelitis) was injected into earthworms, crayfish, yellow perch, goldfish, mud puppies, frogs, garter snakes, turtles, pigeons, guinea pigs, rabbits, and mice. Mice were also inoculated with suspensions of lampblack and fibrin in order to compare the reactions of the leucocytes to an infecting organism and to a particulate matter (lampblack) and a foreign protein (fibrin).

The animals were kept under standard laboratory conditions. Films of blood in circulating body fluids stained by Wright's method

* Contribution from the Department of Zoölogy and the Thomas Henry Simpson Memorial Institute for Medical Research of the University of Michigan.

were studied before the animals were inoculated and at intervals of twenty-four hours after injection of measured amounts of the infecting organism. The types of leucocytes normally found in each species are listed in Tables I and II.

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## EXPERIMENTAL ANIMALS

### *Earthworm* (*Lumbricus terrestris*)

Twenty-four hours after the first injection of staphylococci into the coelom of an earthworm mononucleated cells of the circulating fluid were filled with these organisms (Pl. II, Fig. 1), and twenty-four

## TABLE I

CELLS OF THE CIRCULATING FLUID OF TWO GROUPS OF INVERTEBRATES

The classification of the cells of the coelomic fluid of the earthworm was compiled from a comparative study of the normal cells of *Lumbricus terrestris* and from the description of them by Lim Boon Keng (1895); that for the cells of the crayfish was taken directly from Tait and Gunn (1918), after the normal cells and the description of them by Hardy (1892) and Meinertz (1902) had been studied.

| | | |
|---|---|---|
| *Earthworm* | | |
| Lymphocytes, spherical cells with deeply basophilic coarse granular nucleus | Monocytes (phagocytic, mononuclear), large hyaline cells; inner zone of cytoplasm contains fine eosinophilic granules; peripheral zone contains basophilic granules | Small spherical cells with eosinophilic granules |
| *Crayfish* | | |
| Explosive cells, spindle-shaped cells, and central nucleus | Hyaline thigmocytes (phagocytic); cytoplasm contains a large number of fine granules | Granular thigmocytes, variable in form; oval nucleus and cytoplasm filled with large granules |

hours later the last few segments of the tail region were sloughed off spontaneously. Sections of these discarded terminal segments were filled with bacteria, which were distributed in the coelomic fluid, in the phagocytes, and in the chloragogue cells of that region. Each additional injection was followed by further loss of segments. Some of the animals shed as many as four times.

The regularity with which autotomy of the terminal segments containing phagocytes and bacteria followed injection led to the conclusion that in the earthworm foreign particles are ingested by phagocytic cells of the coelomic fluid and are carried to the posterior segments, which are then discarded. No cellular changes, such as digestion of the bacteria within the cell, are necessary to this animal in resisting infection.

*Crayfish (Cambarus virilis)*

After one day of infection with *S. aureus* the organisms were found in the hyaline thigmocytes of the circulating body fluid. Although these cells were studied in a variety of preparations, in films dried in air and stained with dilute methylene blue, in other films fixed in osmic acid vapor and stained with Wright's stain, in still other films stained with brilliant cresyl blue (vital stain), and in a hanging drop to which the infecting organism had been added, phagocytosis of the organisms was the only change observed. No evidence of the digestion of the bacteria contained in the cells or of any cell inclusions that resemble the granules of the neutrophils of man was visible. Two days after an injection an almost complete absence of phagocytes in the circulating fluid was noted. From this observation we can assume that the species possesses a peculiar mechanism for ridding itself of infecting organisms.

*Yellow Perch (Perca flavescens) and Goldfish (Carassius auratus)*

When staphylococci were first injected into the perch and goldfish the number of leucocytes increased. Continued injections (second, third, and fourth) on successive days maintained this increase in the number of white blood cells, which was also accompanied by phagocytosis (Pl. I, Fig. 2), pycnosis of the nucleus (Pl. I, Fig. 4c), and vacuolization in the cytoplasm. These pathological cells should be compared with the normal neutrophil (Pl. I, Fig. 1). During the course of infection the ingested bacteria underwent a

## TABLE II

### Leucocytes of Various Groups of Vertebrates

The descriptions of the leucocytes are taken from the work of other authors, as follows: for amphibians and mammals, from Scarborough (1930); for perch and goldfish, from Loewenthal (1928, 1930 a and b); for the pigeon, from Kennedy and Climenko (1928). Classification of the leucocytes in the garter snake and the turtle is the result of the author's study of the normal cells and of descriptions of the leucocytes in the literature of the subject.

| Animals infected | Lymphocyte | Monocyte, mononuclear | Neutrophil | Eosinophil | Basophil |
|---|---|---|---|---|---|
| Perch.... | Large and small forms | ..... | Contains fine neutral granules | ..... | ..... |
| Goldfish.. | Normal | Normal | Nucleus may be simple or polymorph | Large cell with eccentric nucleus | ..... |
| Necturus and frog | Normal | ..... | Polymorphonuclear; polynuclear, but may be monolobed or bilobed; contains a few scattered fine metachromatic granules * | Normal | Normal |
| Snake.... | Normal | Large cell with large eccentric nucleus | ..... | Large cell with lens-shaped polar nucleus, spherical or ellipsoidal red granules * | Mononuclear cell |
| Turtle ... | Normal | Large nongranular cell, spherical or oval | ..... | Lens-shaped polar nucleus, large red spherical or ellipsoidal granules * | Large and small varieties |
| Pigeon ... | Small, mononuclear | Large | ..... | Bilobed, with spherical or rod-shaped granules * | Spherical or reniform nucleus |

* Phagocytic.

TABLE II (*Concluded*)

| Animals infected | Lymphocyte | Monocyte, mononuclear | Neutrophil | Eosinophil | Basophil |
|---|---|---|---|---|---|
| Guinea pig | Resembles lymphocytes in man | Some cells contain Kurloff bodies | Pseudoeosinophil, 6–8-lobed nucleus, granules round, slightly basophilic * | Resembles eosinophil in man | Polymorphonuclear |
| Rabbit.. | Resembles lymphocytes in man | Normal | Pseudoeosinophil or amphophile, polymorphonuclear, slight affinity for basic dyes | Polymorphonuclear | Polymorphonuclear |
| Mouse... | Normal | Normal | Polymorphonuclear | Polymorphonuclear | Polymorphonuclear |

* Phagocytic.

gradual change: they increased in size (Pl. I, Fig. 3a), liquefied, completely filled the vacuoles that had formed around them, and became globular masses (Pl. II, Figs. 2–4) in the cytoplasm. As the digested material was absorbed by the cell the globules shrank (Pl. I, Fig. 4b) and changed in staining reaction from a red violet to blue, which gave them the appearance of Döhle bodies (Pl. I, Fig. 5d). The basic-staining areas are not similar to the basophilic granules seen in man during the course of an infectious disease, but they suggest a source of such basophilia.

*Mud Puppy (Necturus maculosus) and Frog (Rana pipiens)*

An increase in the number of leucocytes, phagocytosis, pycnosis of the nucleus, formation of vacuoles in the cytoplasm, and change in the staining reaction from a red violet to a blue were observed in the polymorphonuclear neutrophil (Pl. II, Fig. 6) and in a few mononuclear cells (Pl. II, Fig. 5) as the result of four successive daily injections into the mud puppy and frog. Vacuoles formed around the ingested staphylococci, which liquefied during digestion and created acid areas (Döhle bodies) in the cytoplasm. Nothing ap-

peared to suggest basophilia of the granules of the neutrophils as it appears during a pyogenic infection in man.

*Garter Snake (Thamnophis sirtalis)*

The first injection into the garter snake produced an increase in the number of leucocytes and a slight phagocytosis by the monocytes. Successive inoculations (two and occasionally a third) not only caused an increase of phagocytes, which included mononucleated cells and eosinophils with ellipsoidal granules, but also produced pycnotic nuclei and cytoplasmic vacuoles in the phagocytic cells. Changes in the staining reaction of the ingested bacteria from a red violet to a blue occurred as these organisms underwent digestion.

After the third dose of staphylococci a few inches of the tail of one of the snakes became swollen and discolored. Films made from the blood of this region showed many phagocytes and free bacteria (Pl. III, Fig. 1). The animal recovered without undergoing any further change.

*Turtle (Emys blandingii)*

Four injections administered daily into the coelom of the turtle increased the number of leucocytes and produced vacuoles in the cytoplasm and an almost spherical swelling of the ellipsoidal granules of the eosinophils. Some of these eosinophils were broken while the films were being made, and it was in these cell fragments that staphylococci were observed well embedded near the pycnotic nucleus. Phagocytes were not numerous in the blood films, but there was an increase in the number of the eosinophils with ellipsoidal granules, in the fragments of which were phagocytosed bacteria.

*Pigeon*

The first injection into the pigeon produced a definite leucocytosis which, unlike the condition in other groups, persisted for several days after successive inoculations (five) had been discontinued. In the pigeon the eosinophil with rod-shaped granules is the phagocytic cell, and during the time of infection these granules became so swollen that they gave the cell a "plump" appearance (Pl. III, Fig. 2). The change in shape was accompanied by vacuolization of the cytoplasm and changes in staining reaction from a lighter to a darker blue. The digested material in the swollen granule never quite filled the vacuole as it did in other animals.

*Guinea Pig*

One injection produced an increase in the number of leucocytes. No further change was apparent after a second injection. Basophilia of some of the granules of the pseudoeosinophils (Pl. III, Fig. 3) followed the third injection, and basophilia of a greater number of granules ensued after the fourth. This basophilia of the granules of the pseudoeosinophils of the guinea pig is similar to that of the polymorphonuclear neutrophils appearing in man during the course of an infectious disease.

Two guinea pigs died after receiving extra large doses of unusually virulent *S. aureus*. Blood taken from their hearts showed all the pseudoeosinophils filled with staphylococci.

*Rabbit*

Three subcutaneous injections were given on alternate days to the rabbits. The infection so induced was accompanied by basophilia of the granules of the pseudoeosinophils (Pl. III, Fig. 6), similar to that found in human neutrophils during the course of an infectious disease.

*Mouse* (*Mus musculus*)

Young mice of C57 (black) and dba (dilute brown) varieties from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, were used in this experiment.

In the normal neutrophils of the black mice no granules occur. After two days of infection a few fine slightly basophilic granules appeared in the cytoplasm of the neutrophils. A third day of infection increased the number of basophilic granules of these cells (Pl. III, Fig. 5). No further change could be caused.

In the dilute brown variety of mice two injections produced basophilia of the granules of some of the neutrophils. Basophilia of the granules of a greater number of the neutrophils followed the third injection (Pl. III, Fig. 4). In both varieties of mice this basophilia was similar to that observed in the rat in a series of experiments by the writer (O'Reilly, 1936) and to that found in man during a bacterial infection.

Two groups of mice were inoculated with a sterile suspension of lampblack in saline: one group received three injections at weekly intervals; the other group, the same number but on successive days.

Twenty-four hours after the first injection deposits of this substance were present in the neutrophils (Pl. I, Fig. 6). Similar deposits were observed several days after the inoculations had been discontinued.

A sterile suspension of powdered fibrin (one per cent in physiological salt solution) was also used to inoculate two groups of mice. Two died a few hours after the first inoculation: one a few hours after the second inoculation of fibrin; the other shortly after a third inoculation of the substance. In the blood films made after the injections only an occasional neutrophil was found. Neither basophilia of the granules nor any change in the cytoplasm of the few neutrophils remaining in the circulation was evident.

## DISCUSSION

Results of this investigation of the reaction of leucocytes in a variety of animals to the presence of an infecting organism indicate that in each class of animals studied a particular reaction or set of reactions is induced to protect the animal against bacteria and their metabolic products. In man reaction to infection can be noted by visible changes in the neutrophils. Normal neutrophilic granules become more acid (basophilia of the granules), acid areas (Döhle bodies) appear in the cytoplasm, vacuolization occurs, and the nucleus becomes more compact (pycnotic).

In the animals studied many of these changes, such as the presence of vacuoles and acid areas which resemble Döhle bodies in the cytoplasm and pycnosis, were duplicated. In the lower vertebrates these reactions were preceded by phagocytosis. Basophilia of the granules was limited to mammals, whereas in the invertebrates the cells of the circulating body fluids were actively phagocytic, but presented none of the changes noted in the vertebrates. From these observations we may conclude that the visible mechanisms for resisting the action of infecting organisms in the blood or circulating fluid of the groups of animals under investigation fall into three distinct categories: (1) phagocytosis and excretion; (2) phagocytosis and digestion; and (3) basophilia of the granules of the polymorphonuclear neutrophils or their homologues, the pseudoeosinophils.

Cells of the circulating body fluids of the earthworm defend themselves against bacteria by phagocytosis and autotomy. Cuénot (1897) and Willem and Minne (1899; cited by Lison, 1928) acknowl-

edge the existence of an excretory function of the "amoebocytes" of *Lumbricus* and describe the passage of cells containing particles of injected dye from the coelom into the lumen of the intestine, where they are excreted with the feces. Lison (1928), commenting on the method by which the animal rids itself of tubercle bacilli, says that the organisms are removed by phagocytosis and excretion, and Keilin (1925) finds that, when this animal is infected with parasites, it carries the infecting organisms to the posterior end of the body, where the last few parasite-laden segments are removed by autotomy.

The present study likewise ascribes an excretory rôle to the mononucleated cells, since they engulf the staphylococci that have been injected into the coelom and carry them to the posterior end of the body, where the segments containing them drop off.

The hyaline thigmocytes of the crayfish were definitely phagocytic, but there was no evidence of digestion of the engulfed bacteria; nor were there any cell inclusions that resembled in any way the granules of the neutrophils of man. Although it may be assumed from these observations that the organisms were excreted by the hyaline thigmocytes which contained bacteria after injection and which were definitely phagocytic in a hanging drop, yet these phagocytes never appeared in great numbers in the stained films, and, as a rule, had almost entirely disappeared forty-eight hours after injection. Helen Pixell-Goodrich (1929), in describing an infectious disease of Gammarus, another crustacean, says it is sometimes cured by phagocytosis and autotomy. Though removal of infecting organisms by autotomy is not proved in this study, some evidence has been accumulated which indicates that it occurs.

A second mechanism, that of engulfing and digesting the invading organism, was observed in the lower vertebrates. Leucocytes of the fish, amphibians, and snakes ingested the organisms, formed vacuoles around them, appeared to pour secretions into the vacuoles which digested the bacteria, and then absorbed the soluble material. As digestion and absorption proceeded, the reaction of the digested material changed, so that definitely acid areas which resembled Döhle bodies appeared in the cytoplasm.

An outstanding difference noted in the leucocytes of the snake and in those of the fish and amphibians studied is the inclusion of a granular cell among the phagocytes of the former. Although granules

were present in these cells, there was no suggestion of basophilia of these granules like that seen in man during pyogenic infection.

The ellipsoidal or rod-shaped granules of the eosinophils of the snake, turtle, and pigeon became swollen and assumed an almost spherical shape after injection. This change followed phagocytosis and was accompanied by vacuolization in the cytoplasm, particularly around the ingested organisms, and by digestion and absorption of the engulfed bacteria. The organisms changed in their staining reaction from a red violet to a blue, except in the pigeon, in which the change was from a lighter to a darker blue. As the pigeons recovered from infection the rod-shaped eosinophilic granules that had become swollen lost their spherical shape and became normal in appearance.

Mammals included in this experiment, that is, guinea pigs, rabbits, and mice, responded to injection by a basophilia of the granules of the neutrophils which was similar to the basophilia found in the human neutrophil during the course of an infectious disease. This group showed no evidence, however, of the phagocytosis engendered among the lower vertebrates.

Infection in man is known to produce changes in the nucleus and cytoplasm, and basophilia of the granules of the neutrophils. These same changes were observed in the phagocytic cell of the leucocytes of the vertebrates investigated in this report, although in each group below the mammals they were accompanied by visible phagocytosis. Furthermore, as the engulfed organisms underwent digestion, changes occurred in the staining reaction of the ingested bacteria in the vertebrates but not in the invertebrates. Thus we may conclude that the infecting organism was removed from the invertebrates studied by means other than digestion.

Early in this investigation it became apparent that, if ten or more days were allowed to elapse between the first and the second inoculations, subsequent doses had no visible effect. During the intervening days the animal built up an immunity against the infecting organism. In order to obtain reactions in which the bacteria or their toxins caused definite changes in the cells it was necessary to repeat the inoculations on at least three successive days. That the changes so induced resulted from the injections of *S. aureus* is obvious since the injections of neither lampblack, a particulate matter, nor fibrin, a foreign protein, effected any discernible alteration in the staining properties of the leucocytes.

During the course of the disease some of the infected animals showed signs of it in their appearance and behavior. This was true especially of the snakes, pigeons, rabbits, and mice. Loss of resistance to handling, a drooping appearance, and refusal to eat were common symptoms of infection among the several groups. These clinical changes suggest that the reactions were caused by infection rather than by the injection of inactive particles.

## CONCLUSIONS

1. Large nongranular mononucleated cells of the earthworm engulf infecting organisms and carry them to the posterior end of the body, the last few bacteria-laden segments of which are lost by autotomy.

2. Hyaline thigmocytes of the crayfish phagocytose the infecting organisms and then remove them from the circulating body fluid.

3. Phagocytic leucocytes of perch, goldfish, mud puppy, frog, and snake (monocyte) engulf and digest the injected *S. aureus*, after which changes of the nucleus (pycnosis) and the cytoplasm (vacuolization) are observed. Acid areas resembling Döhle bodies appear in the cytoplasm during the absorption of the digested material.

4. Eosinophils with ellipsoidal or rod-shaped granules engulf the *S. aureus* injected into the snake, turtle, and pigeon. Subsequent changes observed are pycnosis in the nucleus and vacuolization in the cytoplasm. In the snake the eosinophils constitute a second form of phagocyte.

5. Basophilia of the granules in the pseudoeosinophils of the guinea pig and the rabbit and in the neutrophils of the mouse appear after three days of infection with *S. aureus*.

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## EXPLANATION OF PLATE I

FIG. 1. Normal neutrophil of perch

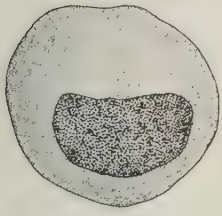
FIG. 2. Neutrophil of perch, showing phagocytosed bacteria and vacuoles in the cytoplasm

FIG. 3. Neutrophil of perch, showing enlargement of the staphylococci in the vacuole (*a*)

FIG. 4. Neutrophil of perch, showing globules in the cytoplasm (*b*) and pycnosis of the nucleus (*c*)

FIG. 5. Neutrophil of perch, showing a few small globules in the cytoplasm resembling Döhle bodies (*d*)

FIG. 6. Neutrophil of mouse, showing phagocytosed lampblack



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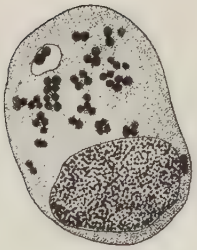

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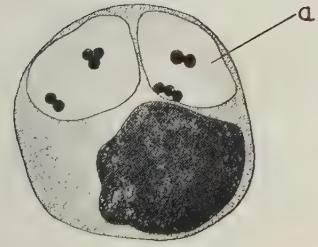


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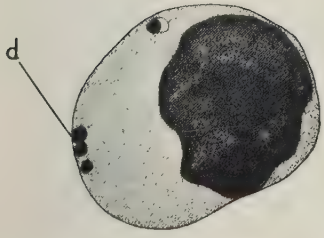


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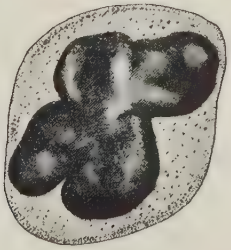

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E. Herbold, del.

## EXPLANATION OF PLATE II

The explanations apply to the white blood cells only.

FIG. 1. Monocyte of earthworm containing phagocytosed bacteria. Twenty-four hours after first inoculation

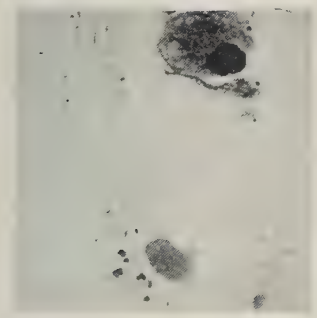
FIG. 2. Neutrophil of goldfish containing phagocytosed bacteria and showing early globule formation. Twenty-four hours after third inoculation

FIG. 3. Neutrophil of perch containing phagocytosed bacteria and showing early globule formation. Twenty-four hours after third inoculation

FIG. 4. Neutrophil of perch showing globule formation. Forty-eight hours after fourth inoculation

FIG. 5. Mononuclear leucocyte of *Necturus* containing phagocytosed bacteria. Twenty-four hours after third inoculation

FIG. 6. Polymorphonuclear neutrophil of frog containing phagocytosed bacteria. Twenty-four hours after first inoculation



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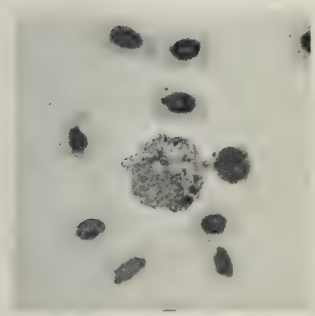

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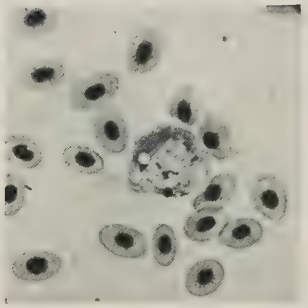

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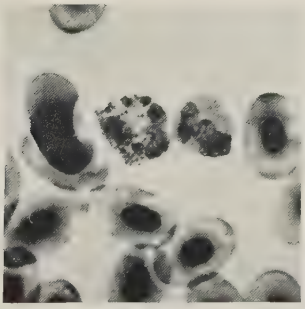

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## EXPLANATION OF PLATE III

The explanations apply to the white blood cells only.

FIG. 1. Monocytes of snake. Blood taken from swollen tip of the tail after third inoculation. Note the appearance of the leucocytes and the number of bacteria

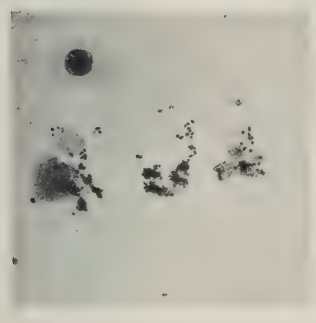
FIG. 2. Eosinophils containing rod-shaped granules of pigeon. Forty-eight hours after fourth inoculation

FIG. 3. Pseudoeosinophil (lower left and upper right) of guinea pig, showing fine basophilic granules. Twenty-four hours after third inoculation

FIG. 4. Polymorphonuclear neutrophils of brown mouse. Twenty-four hours after third inoculation

FIG. 5. Polymorphonuclear neutrophils of black mouse. Twenty-four hours after third inoculation

FIG. 6. Pseudoeosinophils of rabbit showing basophilic granules. Twenty-four hours after third inoculation



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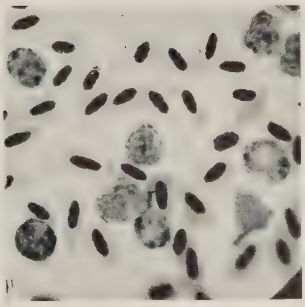

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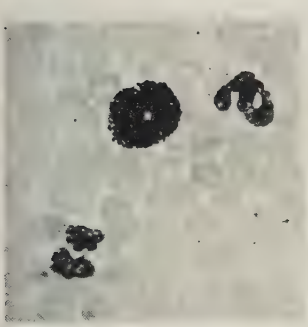

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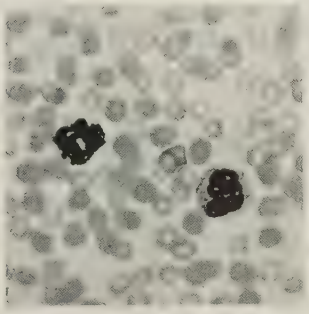

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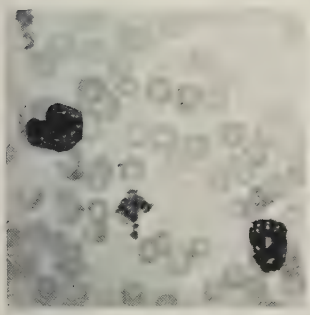

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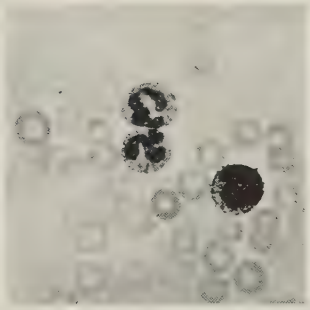

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# THE DISPERSAL OF WILD DUCKS FROM THE W. K. KELLOGG BIRD SANCTUARY, NEAR BATTLE CREEK, MICHIGAN

MILES D. PIRNIE

IN 1931 the author reported on the fall migration of the black duck from northern Michigan as indicated by available records of recaptures of wild individuals trapped and leg-banded at the Munuscong (Munuskong) State Waterfowl Refuge bordering St. Mary's River, approximately 20 miles south of Sault Ste Marie.[1] Opportunity for a similar study in southern Michigan came in 1931, when the author took charge of the W. K. Kellogg Bird Sanctuary of Michigan State College. This sanctuary is located at Wintergreen Lake in northeastern Kalamazoo County, about 50 miles east of Lake Michigan and the same distance north of the Indiana line. It is 250 miles south of the Munuscong Refuge and well within the Mississippi flyway of Lincoln.[2] In this discussion the designation "station area" includes the southwestern corner of the state, twenty counties in all, bordered roughly by a line from Muskegon to Alma, thence south through Lansing and Jackson to the Indiana boundary.

Table I shows the numbers and the species of ducks most commonly trapped and leg-banded at Wintergreen Lake between July, 1931, and December, 1939. They were captured in traps of poultry netting baited with corn and wheat at funnel-shaped entrances. Mallards were taken chiefly at marshy ponds; "blacks" were secured more readily at lake-shore traps. The last column of Table I gives the total and the percentage of recaptures or "returns" for each species up to February, 1940.

By January 31, 1940, 727 bands had been reported, indicating

[1] Pirnie, Miles D., "Fall Migration of the Black Duck from Northern Michigan," *Pap. Mich. Acad. Sci., Arts, and Letters*, 15 (1931) : 485–490. 1932.

[2] Lincoln, Frederick C., *The Waterfowl Flyways of North America*. U. S. Dept. Agric., Circ. 343 (1935).

TABLE I

SUMMARY OF WATERFOWL BANDED AT THE W. K. KELLOGG BIRD SANCTUARY, 1931–39, AND RECAPTURES REPORTED UP TO FEBRUARY, 1940

| Species | Number banded | | | | | | | | | | Reported recaptures |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1931 | 1932 | 1933 | 1934 | 1935 | 1936 | 1937 | 1938 | 1939 | Totals | |
| Black duck (*Anas rubripes*) | 1,194 | 414 | 232 | 129 | 105 | 303 | 630 | 786 | 320 | 4,113 | 516 (12.5%) |
| Mallard (*Anas platyrhynchos*) | 445 | 142 | 41 | 53 | 34 | 99 | 256 | 373 | 303 | 1,746 | 182 (10.4%) |
| Redhead (*Nyroca americana*) | 17 | 33 | 3 | 1 | .. | 23 | 9 | 3 | 2 | 91 | 6 (6.6%) |
| Pintail (*Anas acuta*) | 64 | 7 | .. | .. | .. | .. | 1 | 1 | 7 | 80 | 8 (10.0%) |
| Baldpate (*Mareca americana*) | 58 | .. | .. | 1 | .. | 7 | .. | .. | .. | 66 | 15 (22.7%) |
| Totals | 1,778 | 596 | 276 | 184 | 139 | 432 | 896 | 1,163 | 632 | 6,096 | 727 (12.0%, av.) |

the recapture of over 12 per cent of the 6,096 ducks that were banded between 1931 and 1939. Over 95 per cent of these records came from hunters who shot the birds during the hunting seasons, between September and late December. Spring recoveries were obtained in considerable numbers only for the blacks trapped and shot in Ontario.

Table II summarizes the recoveries of the black duck and the mallard, the only species which were trapped and banded in numbers for nine consecutive years at Wintergreen Lake. In this table the

TABLE II

SUMMARY OF BLACK DUCK AND MALLARD RECOVERIES UP TO FEBRUARY, 1940

| Locality | Black duck | | Mallard | | Totals |
|---|---|---|---|---|---|
| | First year | Later years | First year | Later years | |
| Michigan: | | | | | |
| Station area* | 161 | 150 | 38 | 36 | 385 |
| Outstation area | 13 | 33 | 2 | 4 | 52 |
| Other states | 35 | 74 | 17 | 54 | 180 |
| Canada | .. | 50 | .. | 31 | 81 |
| Totals reported | 209 | 307 | 57 | 125 | 698 |

* The twenty counties of southwestern Michigan.

Michigan totals are segregated from those for other states and Canada. Birds taken within a few weeks or months after banding are listed separately from those captured after a lapse of a year or more.

BLACK DUCKS

Of 4,113 black ducks 516, or 12.5 per cent, have been reported. Michigan recoveries total 357, or almost 70 per cent. Only 46 were shot at a considerable distance from the station area; and four counties (Barry, Allegan, Kalamazoo, and Calhoun) reported 279 bands, more than 75 per cent of the Michigan total! This is not surprising since these banded birds were obviously more concentrated there than elsewhere, and since the many lakes, ponds, and streams of southwestern Michigan are very heavily hunted.

The first-year Michigan recoveries outside the station area include 13 birds which went from 100 to 200 miles north or east a few weeks after they had been banded. These were shot in the northern part of the Lower Peninsula and on Lake St. Clair and Saginaw Bay. Four were killed in central Wisconsin, approximately 200 miles northwest of the point of banding. Examination of Figure 1 and Table III shows first-season recoveries as far away as Missouri, Louisiana, Florida, South Carolina, and Maryland, with a concentration in the Ohio, Michigan, and northern Indiana region.

Later Michigan recoveries in the station area totaled 150 as against 161 the first season; but recaptures elsewhere in Michigan in later years were 33, or more than twice the 13 first-year captures. First-year recoveries in other states totaled 35 as against 74 taken later. Although no banded black ducks were shot in Canada in the season of banding, 50 were eventually reported, of which 32 were from points north of Lake Huron and Lake Superior. Three were killed near Churchill in Manitoba, on the west side of Hudson Bay, and eight on or near James Bay, an average distance of 800 miles north of the banding station. Especially significant are the 22 spring and early-summer records of black ducks far north in Ontario at nesting time. The distribution of the Canadian recaptures is shown by provinces in Table III.

Later occurrences in the United States differ from the first-year spread chiefly in the greater numbers reported from Wisconsin and Minnesota and in the long-distance wandering indicated by the Idaho and Alaska birds.

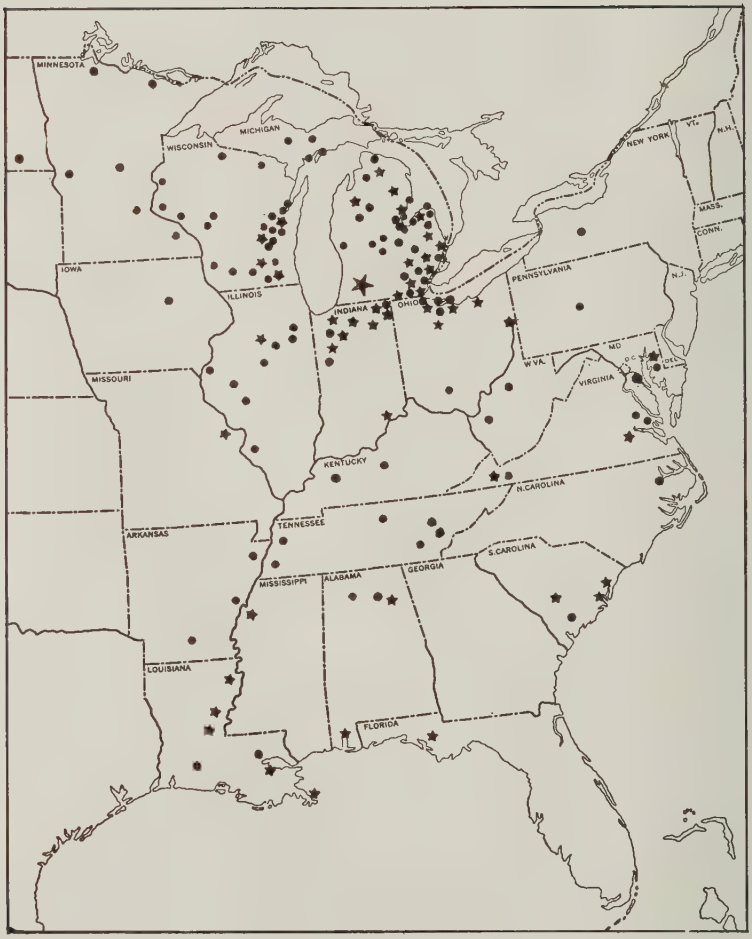


FIG. 1. Black duck recoveries in the United States, not including one in Idaho and 311 recaptured in the station area at Wintergreen Lake (★)

★ Recaptured same year as banded
• Recaptured after one or more years

## TABLE III

Distribution of Black Duck and Mallard Recoveries up to February, 1940

| Province or state | Black duck | | Mallard | | Totals |
|---|---|---|---|---|---|
| | First year | Later years | First year | Later years | |
| Alaska | .. | 1 | .. | .. | 1 |
| Alberta | .. | .. | .. | 2 | 2 |
| Saskatchewan | .. | .. | .. | 2 | 2 |
| Manitoba | .. | 6 | .. | 15 | 21 |
| Ontario | .. | 42 | .. | 11 | 53 |
| Quebec | .. | 2 | .. | 1 | 3 |
| New York | .. | 1 | .. | 1 | 2 |
| Pennsylvania | .. | 1 | .. | .. | 1 |
| Michigan | 174 | 183 | 40 | 40 | 437 |
| Wisconsin | 4 | 21 | 2 | 16 | 43 |
| Minnesota | .. | 6 | 1 | 12 | 19 |
| North Dakota | .. | 1 | .. | .. | 1 |
| South Dakota | .. | .. | .. | 2 | 2 |
| Idaho | .. | 1 | .. | .. | 1 |
| Iowa | .. | 1 | .. | .. | 1 |
| Missouri | 1 | .. | .. | .. | 1 |
| Illinois | 1 | 9 | 1 | 11 | 22 |
| Indiana | 8 | 3 | 2 | 1 | 14 |
| Ohio | 5 | 5 | 1 | 6 | 17 |
| West Virginia | .. | 2 | .. | .. | 2 |
| Maryland | 2 | 1 | .. | .. | 3 |
| Virginia | 2 | 3 | .. | .. | 5 |
| Kentucky | .. | 2 | .. | .. | 2 |
| Tennessee | .. | 6 | 4 | 2 | 12 |
| Arkansas | .. | 3 | 1 | 1 | 5 |
| Texas | .. | .. | .. | 1 | 1 |
| Louisiana | 5 | 2 | 1 | .. | 8 |
| Mississippi | 1 | .. | 1 | 1 | 3 |
| Alabama | 2 | 2 | 1 | .. | 5 |
| North Carolina | .. | 1 | .. | .. | 1 |
| South Carolina | 3 | 2 | 2 | .. | 7 |
| Florida | 1 | .. | .. | .. | 1 |
| Totals | 209 | 307 | 57 | 125 | 698 (12%, av.) |

### MALLARDS

Of 1,746 mallards leg-banded from 1931 through 1939, 182, or 10.4 per cent, had been reported prior to February, 1940. Figure 2 shows the location of all recoveries in the United States, and Table II contrasts early and later recoveries. Table III gives total returns by states and provinces. The Michigan total for later years equals that of the first year, but returns from other states were more than trebled. Of the 182 recoveries Canada turned in 31, or 17 per cent, which is almost double the 9 per cent of black duck returns (only 50 out of 516).

The dispersal of mallards soon after banding is shown by the small stars in Figure 2. Attention is called to the two in Wisconsin and the one in southern Minnesota northwest of the Wintergreen Lake banding station. The Minnesota bird had traveled at least 400 miles to the west and north in eight days! Eastern drift is shown by the two birds shot in the Detroit area and the one at Toledo. Very strangely, of the 15 reported south of Kentucky 10 were first-year recoveries and only five were of later periods. (In later years the total for the United States, excluding Michigan, trebled the first-year kill.) Subsequent travel through Wisconsin, Minnesota, and the prairie provinces is shown in Figure 2 and Table III. Significant are the 15 Manitoba recoveries and the 11 Ontario ones. The two Saskatchewan records are the farthest west. Several birds were shot north of Lake Winnipeg in Manitoba, also two at the south shore of Hudson Bay and one near Montreal. All four of the later recoveries in Michigan away from the station area were from the marshes of Saginaw Bay. (In contrast, many of the later recoveries of black ducks were from interior waters as well as from Saginaw Bay, Lake St. Clair, and the Erie marshes.)

### OTHER SPECIES

Of 91 redheads banded six have been reported in later years, all on an east–west axis, as follows: South Dakota, 1; Detroit area, 1; southern Ontario, 2; and Maryland, 2.

Eighty pintails were leg-banded, as indicated in Table I. The eight recoveries were widely scattered. There were two first-year kills near the station; for later periods the recoveries are: Wisconsin, 2; Kentucky, 1; Tennessee, 1; North Carolina, 1; South Carolina, 1.

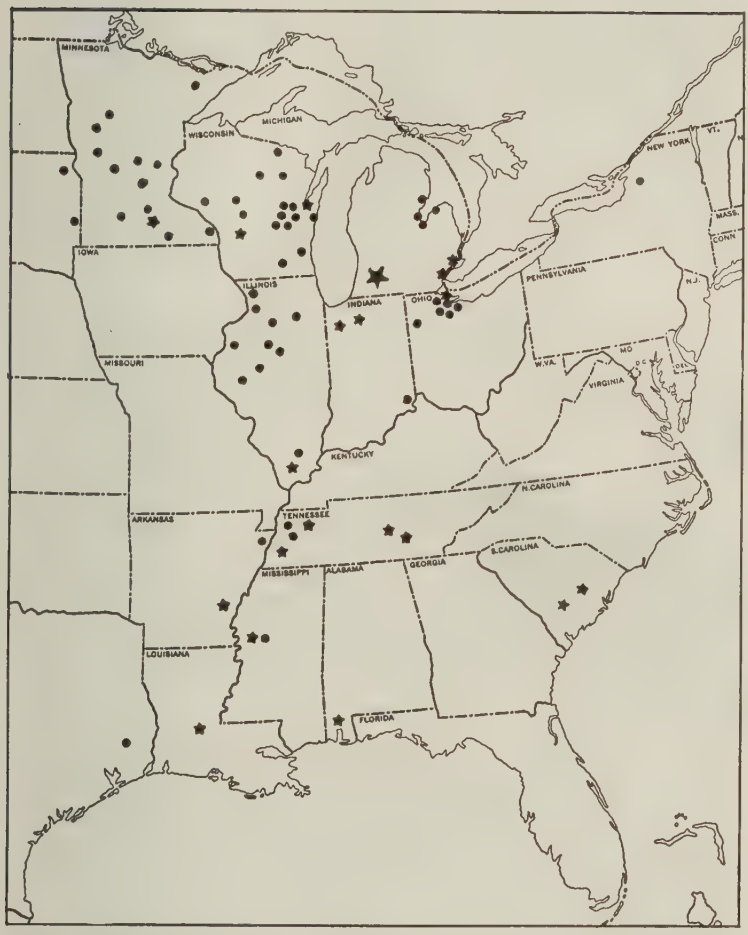


FIG. 2. Mallard recoveries in the United States, not including 84 recaptures in the station area at Wintergreen Lake (★)

★ Recaptured same year as banded

• Recaptured after one or more years

Again a southeast drift across the central and southeastern states is suggested.

The 66 baldpates gave a total of 15 returns. First-season dispersal includes one reported in southern Wisconsin and one from Long Island, New York. Among later recoveries are those from: Lake Athabaska in Alberta, 2; Nebraska, 1; Wisconsin, 2; Michigan, 5; Virginia, 2; and North Carolina, 1. Again the diagonal flight is indicated — southeast and northwest. The 23 per cent kill reported strongly suggests the importance of shooting as a mortality factor.

SUMMARY

1. In their fall dispersal from southern Michigan some black ducks and mallards travel as far as 200 or more miles west, north, or east. A mallard was shot in southern Minnesota on October 22, 1932, only eight days after it had been banded at Wintergreen Lake. Two mallards and four black ducks were killed in Wisconsin and numbers of black ducks have been taken in northern Michigan counties the same fall as they were banded.

2. The southward drift of black ducks and mallards from southern Michigan resembles the pattern of black duck dispersal from northern Michigan, but mallards seem to scatter less widely over the southeast. Recoveries in Louisiana are quite frequent, if the gradual dilution of banded individuals among unbanded birds is taken into consideration.

3. The black ducks marked at Wintergreen Lake scatter widely over the Mississippi and Atlantic flyways, from northern Manitoba and Ontario south to Louisiana and South Carolina, but apparently avoid the northeastern states.

4. Recovery of 22 banded black ducks in northern Ontario in spring and early summer strongly indicates that this is the nesting home of many of the individuals leg-banded in this study.

5. A black duck shot in Idaho and another on the west coast of Alaska confirm the western extension of range credited to this species by numerous observers in recent years.

6. The mallards were reported chiefly from the Mississippi flyway, migrating from the prairie provinces down across Minnesota, Wisconsin, and Illinois to Louisiana by a gradually narrowing route. Few seem to reach the Atlantic coast. The 30 captures at points in Canada north and west of Michigan indicate Canadian nesting by

many of the mallards which were leg-banded in fall at Wintergreen Lake.

7. The very limited banding of baldpates and redheads has yielded some evidence of migration along a northwest–southeast diagonal route. The pintail returns indicate a more general scattering over the south and east.

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# THE INHERITANCE OF A COLOR VARIATION IN THE CODLING MOTH, *CARPOCAPSA POMONELLA* LINNÉ

PAUL L. RICE

## INTRODUCTION

AN EXAMINATION of the publications in the field of economic entomology during the past fifteen years will probably show that more space has been given to the discussion of the codling moth, *Carpocapsa pomonella* Linné, than to any other insect. Most of these numerous articles and bulletins have to do with either the biology or the control of this troublesome orchard pest. The present discussion is a less serious one. The author has concerned himself with a study of the genetical behavior of certain codling moths that show a definite color variation.

While occupied with entomological work for the Idaho Agricultural Experiment Station the writer noticed that some specimens of the codling moth were distinctly different in their coloration from the common type. This color variety had been observed by entomologists for a number of years. The problem of investigating the possible genetical relationship of these two strains was undertaken. The work was begun in 1927 and was continued intermittently, as a minor project, through 1934. At that time it was hoped that further experiments could be carried on to confirm some of the results obtained, but a change of residence prevented this. Although the results reported below are not so full as might be desired, it is felt that they are sufficiently conclusive to warrant their publication.

## DISTRIBUTION AND FREQUENCY OF OCCURRENCE OF LIGHT MOTHS

The usual color of the codling moth is dark gray, with spots of a bronze shade, whereas the light strain is tan, spotted with brown. Observations made in southwestern Idaho in 1934 indicated that approximately six per cent of the population were of the light-colored

variety. This estimate was based upon counts of 9,017 moths, of which 529 were found to be tan. An earlier sample, taken in a different community within the same general region, showed that the percentage of light moths there was twice as high. So far as it was possible to determine, the light variety occurs in greater frequency in southwestern Idaho than elsewhere. In northern Idaho the light specimens are much scarcer, and in central Washington they appear so infrequently that they are almost regarded as an oddity by the entomologists. While the writer was in Delaware he examined several thousand moths, but saw none of the light variety. Observations in other states in the East and in the Middle West, together with information gained from entomologists living in these regions, have led to the belief that the light strain is unknown there.

## PROCEDURE AND METHODS

Initial experiments showed that, when light moths crossed with light, the offspring were all of the parental color, but a small percentage of light moths appeared among the progeny of dark parents. These observations indicated that the color variation had a genetical significance, and hence it could not be accounted for by variations in environmental conditions. After this, further experimental crosses were carried out, light being mated with light, dark with dark, and dark with light. Later, when the results had justified the tentative assumption that dark was the dominant characteristic and light the recessive, heterozygous dark moths were obtained by choosing the dark progeny of light $\times$ dark crosses. Matings were produced among these supposedly heterozygous dark moths and also between the light and the heterozygous dark strains.

The rearing methods used were similar to those employed by a number of workers who have investigated the life history of the codling moth. Adults were confined in battery-jar cages and were allowed to oviposit upon apple leaves. Apples were provided for the larvae to feed upon. Mature larvae were induced to enter corrugated paper strips, where they spun their cocoons and passed through the pupal stage. The colors of the emerging adults were recorded, and they were segregated for additional breeding experiments. Special care was taken in handling all stages in order to prevent contamination of the cages by strains of the insect other than the ones desired.

When a single pair of moths was confined separately within a cage, poor mating results were obtained. Hence it was necessary to place several pairs together in making crosses.

### RESULTS OF THE MATINGS

From the matings in which dark moths, selected at random from the population, were used, 146 progeny were reared to maturity. Of these, 134 were dark and 12 were light. One hundred and fifty-eight moths were obtained from the crosses of light × light. All but one were light in color. Even though great care was taken to prevent mixing of the stock in the various cages, there was still a possibility that a small larva from an unknown strain might have gained entrance to one of the feeding cages containing the larvae of light moths. This would account for the appearance of the dark moth.

One hundred and forty-three offspring were reared from parents assumed to be heterozygous dark. Of this number 31, or 22 per cent, were light.

Only one backcross, involving the mating of heterozygous dark moths with light moths, was made. From this cross 30 offspring were obtained, 60 per cent of which were dark and 40 per cent light.

### DISCUSSION AND CONCLUSIONS

Since dark gray is the common coloration of the codling moth and since the tan variety is apparently limited in its distribution to the Pacific Northwest, it is logical to assume that the light strain has arisen as a mutation from the dark.

Experimental evidence indicates that a single factor is involved in color determination and that the dark characteristic is dominant over the light. The facts that a small percentage of light moths appeared among the offspring of dark × dark matings and that light moths produced light offspring only (the one discrepancy noted, in view of other evidence, being accounted for as an error in technique) support this view. The progeny of crosses in which heterozygous individuals were mated among themselves, where a proportion of 25 per cent light to 75 per cent dark would be expected theoretically, showed a color distribution of 22 per cent light to 78 per cent dark. Since the total offspring reared from such crosses was only 143, these results would be considered reasonably close to the theoretical values. In the backcross, in which the expected $F_1$ results would be 50 per

cent light to 50 per cent dark, the observed results were 40 per cent light to 60 per cent dark. Again the variation from the expected ratios can be explained on the basis of the small population dealt with. These data, when considered together, give strong support to the belief that the factors involved are a simple dominant and a recessive.

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# THE MOVEMENTS OF MARKED FISH IN DOUGLAS LAKE, MICHIGAN *

IMMANUEL A. RODEHEFFER

THIS study of the movements of fish in Douglas Lake, Cheboygan County, Michigan, is the outgrowth of other investigations carried on in the lake by the writer during two previous summers. In the summer of 1937 ten experimental brush shelters were installed and studied at Grapevine Point in Douglas Lake (see map, Fig. 1) to determine to what extent fish of various species and sizes would use such devices as warm weather habitats (Rodeheffer, 1939). In July and August of 1938 the same shelters were kept under continued observation to discover if the same species and sizes of fish would again be found using the structures and to obtain further evidence on the differences in the fish population utilizing the artificial covers by day and by night (Rodeheffer, 1940). To ascertain how rapidly and to what extent shelters would become repopulated if all fish were removed, four more shelters were built and placed in North Fishtail Bay in July, 1938.

These investigations during the summer seasons of 1937 and 1938 in the eastern end of Douglas Lake showed that:

1. If fish shelters are installed on a practically barren shoal, such devices will attract fish (Rodeheffer, 1939).
2. There is a great diurnal fluctuation of fish populations within a given area (Rodeheffer, 1940).
3. As fish are removed from a part of a lake where protection is offered, others will repopulate such regions (Rodeheffer, 1940).

Primarily in connection with the studies of the utilization of the brush shelters by fish many game fish were marked at Grapevine Point in the summer months of 1937 and 1938 and at Hook Point,

* Contribution from the Institute for Fisheries Research of the Michigan Department of Conservation and the Biological Station of the University of Michigan.

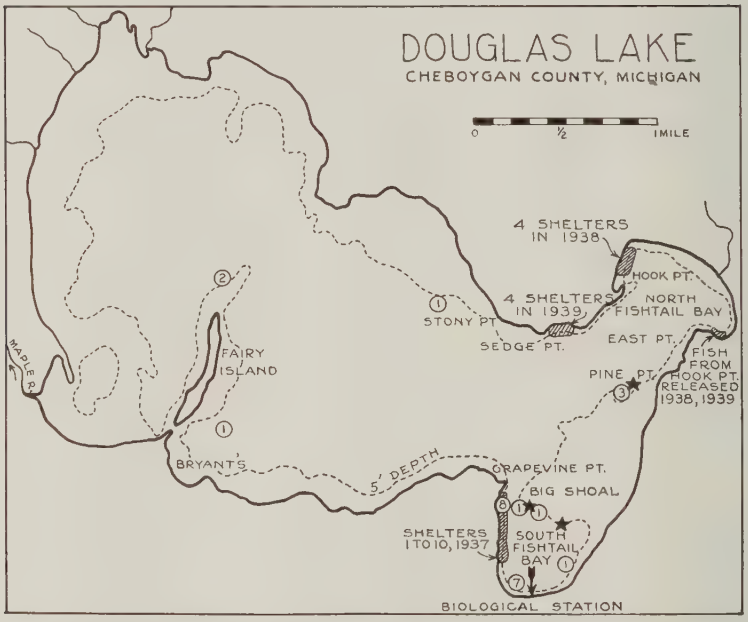


FIG. 1. Map of Douglas Lake, Michigan, showing points where fish were marked, released, and recaptured in a study of their movements. Stars mark the locations of Fyke net settings. Circles indicate the places where planted small-mouthed bass were recaptured; the figures within the circles give the number of bass retaken

North Fishtail Bay, in August, 1938. As the main focus of interest shifted to an analysis of fish movements, marking was continued at these locations and at other points in Douglas Lake in 1939. Small-mouthed bass transported from Lake Michigan and planted in Douglas Lake were marked before their release. In order to add to the little that is known of the wanderings of game fish in a lake, the data on the dispersion of these marked fish as determined by recaptures are presented in this paper.

A better knowledge of the movements of fish is one of the major problems that confront the fisherman and the scientist. "Where are the fish today?" is a stock phrase of the sportsman, and even the most experienced angler will excuse a poor day's fishing by saying that the fish have left his favorite fishing bed.

The practical fisheries manager is especially concerned with the distribution of fish. Should fish be planted at one convenient spot, or would the added expense of stocking fish at several points on a lake be justified? Is it possible that the condition in our lakes is analogous to that found in the deer country — starving fish in one part of a lake and an abundance of food in another? Will some sections be overfished while others are not fished at all? Does the installation of artificial covers make the taking of fish so easy as to deplete the stock? These are only a few of the questions that may be more adequately answered by a knowledge of the actions of fish.

To the fisheries biologist information on the shifting location of fish is essential in life-history and migration studies. What places are sought out for breeding by different species, and do they vie with each other for spawning grounds? Do they seek feeding areas where the young of certain species congregate?

Some insight into the movements of fish may be of value to general biologists and to specialists in fields other than fisheries research. In his study of the ecology of any organism the biologist will be aided by knowing where all fish are to be found at different times in a body of water. The possibility of racial segregation or even differentiation within a lake is another general problem in which such knowledge is of prime importance. The limnologist, too, may find that this information will throw new light on the various biological aspects of his studies, such as those on productivity.

ACKNOWLEDGMENTS

Again I should like to express my appreciation to Dr. A. S. Hazzard, director of the Institute for Fisheries Research, for assistance in planning this work, and to Dr. George R. La Rue, director of the Biological Station, for suggestions and equipment. As in former years, I owe a great debt to Dr. Carl L. Hubbs for guidance in this investigation and help in the preparation of the report. Thanks to the officials of the Michigan State Civilian Conservation Corps, labor was again furnished by selected enrollees.

## METHODS OF MARKING FISH

1. *Fin clipping.* — The pelvic fins and pectoral fins were clipped on certain fish. Removing different fins made it possible to designate the fish of different locations distinctively. Regeneration of the

clipped fins was prevented by removing them at the base, with a pair of curved manicure scissors.

2. *Jaw-tagging.* — Numbered metal jaw tags (Shetter, 1936) were placed on the mandible maxillary or premaxillary. This method permits the tracing of the movements of individual fish.

3. *Silver wire.* — Fine silver wire was twisted around the right or left mandible of a small number of fish. The specimen was grasped in the left hand and held against the body. After a piece of the silver wire, cut to the proper length, had been inserted around the mandible, the ends were securely held, close to the fish's jaw, by the thumb and forefinger of the left hand, so that a loose loop was formed as the ends were twisted together by tagging pliers in the right hand and then bent back against the twisted part of the wire to prevent their catching on the net or other objects.

Rock bass (*Ambloplites rupestris*), pumpkinseeds (*Lepomis gibbosus*), and yellow perch (*Perca flavescens*) were marked by all three methods. Small-mouthed bass (*Micropterus d. dolomieu*) and large-mouthed bass (*Huro salmoides*) were fin-clipped in 1937 and tagged with metal jaw tags in 1938 and 1939. Northern pike (*Esox lucius*) were tagged with metal jaw tags.

### METHODS USED TO RECAPTURE MARKED FISH

Several different methods were used to get returns on marked fish in Douglas Lake:

1. A 140-foot bag seine, made of $\frac{1}{4}$-inch mesh in the bag, of $\frac{3}{8}$-inch mesh next to the bag, and of $\frac{7}{8}$-inch mesh at the ends of the wings, was used for all seining. For deep shelters and weed beds a piece of net 6 × 80 feet was attached to the top of the center section of the seine to permit effective work in deeper water.

2. Posters were placed around the lake at the various resort centers asking fishermen to report any marked fish.

3. Two fyke nets, the property of the Biological Station, were used to catch fish on several drop-offs away from shore.

### EXPERIMENTS AT GRAPEVINE POINT

In 1937 ten experimental fish shelters were built and placed on the shoal of Douglas Lake between Grapevine Point and the boat-

house of the University of Michigan Biological Station (see Fig. 1). A description of the area and of these constructions is given in a former paper (Rodeheffer, 1939). The first brush pile, called shelter 1, was placed just south of the little bay formed by Grapevine Point, in about 6 feet of water. All other constructions located on the 6-foot contour were numbered consecutively to the south. They were set approximately 80 feet apart except where control areas were designated. Here the distance between the installations was about 180 feet. Control areas established between shelters 2 and 3, 4 and 5, 6 and 7, 8 and 9, and south of shelter 10 were sections similar to the locations where fish protections were installed. This arrangement separated shelters 1 and 10 by approximately 1,100 feet. The devices were constructed and placed so that they could be pulled out to determine to what extent they were used by fish and the size and species of game fish found in them. Before the brush piles were removed the 140-foot bag seine was laid around them in the form of a semicircle. When the shelters were pulled shoreward the net was carefully drawn along behind, catching the fish in or near the artificial covers. The captured fish were identified, checked for previous markings, measured, and released.

In 1937, to discover if fish used the brush devices as summer habitats, the pelvic fins were clipped on all game fish before they were freed. On August 2, 4, and 6 the right pelvic fin was removed on all captured game fish; on August 23, 24, and 25 the left pelvic fin was clipped on the seined fish. Fish that were recaptured on August 2, 4, and 6 were recorded as being marked, then returned to the lake. When any fish with the right pelvic fin missing was retaken on August 23, 24, and 25, the left pelvic was also excised. Fish caught with both pelvic fins clipped were so recorded and set free. Table I presents the data of the species and numbers of fish with one or both pelvic fins removed. In 1938 the ten shelters were again pulled from the water and the fish counted, identified, checked for markings, and measured. On July 12 and 13 shelters 1, 2, and 10 were taken out and all game fish of sufficient size were tagged with metal jaw tags. Thirty-eight small-mouthed bass taken from all ten shelters and in four control areas between July 12 and August 26 were tagged.

To economize on time and tags in 1938 it was decided to clip the

## TABLE I

NUMBER OF FISH, BY SPECIES, MARKED BY DIFFERENT METHODS AT GRAPEVINE POINT, DOUGLAS LAKE, AND PERCENTAGE OF THEM RETAKEN (ALL AT GRAPEVINE POINT) IN 1937, 1938, AND 1939

The percentages are based on all fish recaptured, whether once or more than once, because many of the fish were not marked so as to be individually recognizable and because the recaptured fish were released alive.

| Date of original markings and recoveries | Rock bass | Yellow perch | Pumpkin-seeds | Small-mouthed bass | Large-mouthed bass | North-ern pike | All species |
|---|---|---|---|---|---|---|---|
| Number marked Aug. 2–25, 1937, by clipping one or both pelvic fins.... | 1,320 | 663 | 262 | 210 | 22 | ... | 2,477 |
| Percentage recovered | | | | | | | |
| 1937..... | 38.5 | 16.9 | 21.8 | 13.3 | 4.5 | ... | 28.5 |
| 1938..... | 13.4 | 3.3 | 1.5 | ... | ... | ... | 8.2 |
| 1939..... | 4.4 | 0.3 | ... | ... | ... | ... | 2.4 |
| Number marked July 12–Aug. 26, 1938, by clipping one or both pectoral fins..... | 582 | 566 | 95 | ... | ... | ... | 1,243 |
| Percentage recovered | | | | | | | |
| 1938..... | 15.3 | 13.3 | 6.3 | ... | ... | ... | 13.7 |
| 1939..... | 16.2 | 2.7 | 1.1 | ... | ... | ... | 8.9 |
| Number marked July 12–Aug. 26, 1938, with numbered jaw tags... | 185 | 137 | 17 | 38 | ... | 1 | 378 |
| Percentage recovered | | | | | | | |
| 1938..... | 13.0 | 13.1 | 11.8 | 47.4 | ... | 200.0 | 16.9 |
| 1939..... | 3.8 | ... | ... | ... | ... | ... | 1.9 |
| Number marked July 24–Aug. 16, 1939, by silver wire..... | 134 | 76 | 34 | ... | ... | ... | 244 |
| Percentage recovered, 1939 | 42.5 | 5.3 | 2.9 | ... | ... | ... | 25.4 |
| Number marked July 11–Aug. 16, 1939, with numbered jaw tags... | 71 | 66 | 51 | 20 | 6 | 1 | 215 |
| Percentage recovered, 1939 | 50.7 | 3.0 | 5.9 | 30.0 | 33.0 | ... | 22.8 |
| Total number marked by all methods 1937, 1938, and 1939..... | 2,292 | 1,508 | 459 | 268 | 28 | 2 | 4,557 |
| Percentage recovered | | | | | | | |
| Same year..... | 31.2 | 14.0 | 15.0 | 19.4 | 10.7 | 100 | 23.1 |
| Second year *..... | 13.3 | 2.8 | 1.3 | ... | ... | ... | 7.8 |
| Third year *..... | 4.4 | 0.3 | ... | ... | ... | ... | 2.4 |

* In percentage of marked fish then potentially available for recapture.

pectoral fins on all game fish except small-mouthed bass, large-mouthed bass, and northern pike at shelters 1, 4, and 10 at Grapevine Point. After July 13 all rock bass, yellow perch, and pumpkinseeds taken in the net had the right pectoral fin removed at shelter 1, the left pectoral at shelter 10, and both pectorals at shelter 4. All fish tagged or fin-clipped in 1938 were returned to the water at the

shelter after it had been replaced. The number and species of fish marked by the different methods in 1938 are given in Table I.

In 1939 all bass and northern pike were again tagged. At shelter 1 the right mandible of rock bass, yellow perch, and pumpkinseeds of sufficient size was marked with silver wire. Fish from shelter 10 were wired on the left mandible. At shelter 4 all fish were tagged with metal jaw tags. Table I details the number and species of fish marked in 1939 also.

### RECAPTURES OF MARKED FISH AT GRAPEVINE POINT

Marking the fish caught at Grapevine Point made it possible to keep a record of the number of fish that were recaptured in 1937, 1938, and 1939. Fish were retaken (1) with pelvic fins missing — fish marked in 1937; (2) with metal jaw tags or pectoral fins clipped — fish marked in 1938; (3) with metal jaw tags or wired jaws — fish marked in 1939.

Table I gives the percentage of the fish recaptured each year, grouping them according to the different marking devices. The percentage of recovery for fin-clipped and wired fish was probably increased by reason of the fact that some fish were netted more than once. For the tagged fish, which were marked so as to be individually identified, it was found that of the fish tagged in 1938 at Grapevine Point one rock bass, two perch, and five small-mouthed bass were twice recovered in 1938, and that the one sunfish and the one northern pike were both taken a second time in that year. Of the rock bass tagged in 1938 three were again secured in 1939, and one of these was recorded five times. Of the fish individually tagged in 1939 six rock bass were recovered twice and five were caught thrice. None of the fish of the other species tagged were reseined more than once at Grapevine Point. In 1937 and 1939 a high percentage of retakes of the fish that were tagged the same year is indicated. Table I shows a declining number taken in 1938 and 1939 of those marked in 1937. A smaller percentage of the fish tagged in 1938 was retaken in 1938, with an unexplained increase in 1939 in the number of rock bass recaptured. There is also some difference in the percentages of re-netted fish that were tagged, fin-clipped, or marked with silver wire. The number of returns by each marking method is, however, probably too small to warrant any conclusions as to which procedure is the most satisfactory.

THE MOVEMENTS OF FISH AT GRAPEVINE POINT

Tagging and fin-clipping in 1938 and 1939 at Grapevine Point made it possible to trace the movements of fish between shelters during the two summers. Table II specifies, for fish which were retaken, how far they had wandered from the point of original capture (or from the point of last capture in the case of the fish tagged for individual recognition) during the first and second years. It thus includes records for fish which are known to have been netted a number of times, and an exaggerated amount of traveling may be

## TABLE II

MOVEMENTS OF MARKED GAME FISHES AT GRAPEVINE POINT, DOUGLAS LAKE, AS DETERMINED BY THE DISTANCE IN FEET BETWEEN POINTS OF CAPTURE AND OF RECAPTURE

For fish not individually marked it was necessary to assume that the movement was always from the point of original capture and marking. This circumstance may have exaggerated the amount of wandering indicated.

| Species, year of recapture (and number of records) | Known distance traveled, in feet | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 80 | 170 | 260 | 340 | 430 or 520 | 600 or 690 | 780 or 860 | 1,040 or 1,120 |
| Rock bass | | | | | | | | | |
| First year (206) | 105 | 30 | 1 | 10 | 27 | 13 | 7 | 12 | 1 |
| Second year (101) | 46 | 14 | .. | 10 | 10 | 10 | 1 | 3 | 7 |
| Perch | | | | | | | | | |
| First year (98) | 39 | 32 | 7 | 3 | 6 | 1 | 4 | 2 | 4 |
| Second year (15) | 13 | 2 | .. | .. | .. | .. | .. | .. | .. |
| Pumpkinseed | | | | | | | | | |
| First year (12) | 1 | 4 | 1 | 2 | 2 | 2 | .. | .. | .. |
| Second year (1) | ... | .. | .. | 1 | .. | .. | .. | .. | .. |
| Small-mouthed bass | | | | | | | | | |
| First year (24) | 4 | 9 | 4 | 2 | 3 | 2 | .. | .. | .. |
| Second year (0) | ... | .. | .. | .. | .. | .. | .. | .. | .. |
| Large-mouthed bass | | | | | | | | | |
| First year (2) | ... | 2 | .. | .. | .. | .. | .. | .. | .. |
| Second year (0) | ... | .. | .. | .. | .. | .. | .. | .. | .. |
| Northern pike | | | | | | | | | |
| First year (2) | 2 | .. | .. | .. | .. | .. | .. | .. | .. |
| Second year (0) | ... | .. | .. | .. | .. | .. | .. | .. | .. |
| All species | | | | | | | | | |
| First year (344) | 151 | 77 | 13 | 17 | 38 | 18 | 11 | 14 | 5 |
| Second year (117) | 59 | 16 | 0 | 11 | 10 | 10 | 1 | 3 | 7 |

indicated, since it is possible that some fish not individually marked swam to another shelter, making that cover a permanent habitat at which they may have been taken several times. For each such recapture the distance from the shelter where the fish had first been marked and released was recorded. The maximum number (five) of changes noted for a single fish were made by a rock bass which was tagged at shelter 2 on July 13, 1938, but not reseined in 1938. In 1939, on July 19, it was caught at shelter 2 (and therefore recorded as not having traveled). On July 28 it was taken 260 feet away, at shelter 4. On August 2 the same fish was recovered at shelter 2, so that 260 feet of travel was again listed. On August 4 it was seined at shelter 4, after another known shift of 260 feet. On August 16 the fish was caught again, this time at shelter 1 (340 feet from shelter 4). The sum of the known movements of this rock bass was 1,120 feet, but all the back-and-forth roaming, so far as is known, was within a distance of 340 feet. Table II indicates that the meanderings of fish are restricted within a given area. The larger number of fish were found to remain near the place of original capture. Rock bass show the greatest tendency to stay at (or to return to) the same refuge the second year. Yellow perch, usually considered a free-swimming fish, seem to live, during the first year, near the shelter where first captured. The few retaken in the area the second summer may merely indicate random swimming. Small-mouthed bass, although few were marked, showed restricted travel in one season and no indications of a return to the same region the second year. Pumpkinseeeds, large-mouthed bass, and northern pike were so few that conclusions regarding their wanderings are not warranted. It is most striking that not a single fish of the 4,557 marked at Grapevine Point from 1937 to 1939 was recovered at any other point in the lake. Lack of returns from sportsmen fishing in other parts of Douglas Lake might have been expected, as there is little angling here for pan fish, but a large amount of collecting was done around the eastern part of the lake, not only by the writer but also by other workers at the Biological Station, all of whom were looking for the marked fish. At Grapevine Point 1,051 recaptures were recorded during the year of marking, 320 during the following year, and 60 during the third year. What happened to the marked fish that were never renetted can only be guessed at. The rate of mortality among marked fish is not known but only seven marked rock bass and three marked perch

were found dead during the three years despite the fact that the beach around South Fishtail Bay was repeatedly traversed during all three summers. In 1939 a daily check was made of the entire shore from Grapevine Point to Pine Point (Fig. 1).

No doubt many fish, including, perhaps, some which had been marked, were eaten by gulls.

### EXPERIMENTS AT NORTH FISHTAIL BAY

To gather more information regarding the movements of fish four additional brush shelters were constructed and placed in Douglas Lake, just north of Hook Point, in North Fishtail Bay, in July of 1938 (Rodeheffer, 1940). These shelters were removed and the fish caught at intervals between August 3 and 22, 1938, and between July 20 and August 9, 1939. In order to determine how rapidly and to what extent such shelters become repopulated all fish netted from these structures were carried a straight-line distance of about 0.6 mile across North Fishtail Bay, to be released in the small sheltered cove which lies to the east of East Point (Fig. 1). All game fish of sufficient size were tagged with metal jaw tags before being set free to see if any would return to the artificial covers. These operations also provided a further means of checking on the movements of fish marked at Grapevine Point and of those liberated at East Point. There were few returns from the fish that were tagged at Hook Point and put back into the water at East Point, but the individuals taken at various locations (as specified in Table III) suggest that these fish reacted similarly to planted fish in moving around somewhat at random. The fact that most of the recoveries from the shelters at Hook Point were of rock bass may be explained by the preference of rock bass for brush shelters (Rodeheffer, 1939, 1940). As indicating the limited natural movements of fish in the lake, it may again be pointed out that nineteen hauls, with a 140-foot seine, in 1938 and ten hauls in 1939 from shelters and control areas at Hook Point failed to capture any of the 4,557 marked fish released at Grapevine Point in 1937, 1938, and 1939.

The return of fish from East Point to Hook Point might be regarded as evidence of homing behavior, but no such conclusion is warranted by the facts at hand. Hook Point forms a natural trap and was a favorable and well-populated fish habitat even before the brush shelters were installed.

## TABLE III

KNOWN MOVEMENTS OF FISH CAPTURED AND TAGGED AT HOOK POINT, DOUGLAS LAKE, AND RELEASED AT EAST POINT

Recoveries are indicated for the several points in Douglas Lake where collections were made in the given year.

| Date of tagging and date and place of recovery | Rock bass | Yellow perch | Pumpkin-seeds | Small-mouthed bass | Large-mouthed bass | North-ern pike | All species |
|---|---|---|---|---|---|---|---|
| Number tagged in 1938.. | 205 | 160 | 70 | 20 | 6 | 3 | 464 |
| Recoveries in 1938 | | | | | | | |
| Hook Point..... | 16 | 3 | ... | 1 | .. | .. | 20 |
| Grapevine Point... | ... | ... | ... | .. | .. | .. | ... |
| Pine Point..... | 1 | ... | ... | .. | .. | .. | 1 |
| Recoveries in 1939 | | | | | | | |
| Hook Point..... | 10 | 1 | ... | .. | .. | .. | 11 |
| Sedge Point..... | ... | ... | ... | .. | .. | .. | ... |
| Pine Point..... | ... | ... | ... | .. | .. | .. | ... |
| South drop-off, Big Shoal..... | ... | ... | ... | .. | .. | .. | ... |
| East drop-off, South Fishtail Bay..... | ... | ... | ... | .. | .. | .. | ... |
| Grapevine Point... | 1 | ... | ... | .. | .. | .. | 1 |
| Number tagged in 1939.. | 115 | 98 | 74 | 9 | 5 | 2 | 303 |
| Recoveries in 1939 | | | | | | | |
| Hook Point..... | 4 | 1 | 1 | .. | .. | .. | 6 |
| Sedge Point..... | 2 | ... | ... | .. | .. | .. | 2 |
| Pine Point..... | ... | ... | ... | 1 | .. | .. | 1 |
| South drop-off, Big Shoal..... | ... | ... | ... | .. | .. | .. | ... |
| East drop-off, South Fishtail Bay..... | ... | ... | ... | .. | .. | .. | ... |
| Grapevine Point... | ... | ... | ... | .. | .. | .. | ... |
| Total number tagged in 1938 and 1939... | 320 | 258 | 144 | 29 | 11 | 5 | 767 |
| Total number recaptured in 1938 and 1939. | 34 | 5 | 1 | 2 | .. | .. | 42 |

### OTHER ATTEMPTED RECOVERIES

Investigations in 1937 and 1938 showed slight movement of fish in Douglas Lake. In attempting to determine how restricted the wandering is (and also to discover if fish of different sizes inhabit shelters at different depths), four ladder-shaped shelters (Hubbs and Eschmeyer, 1938, pp. 74–80, figs. 16–19) were made; two were placed in 5 feet of water, and two at a depth of 10 feet. These were installed in 1939, just east of Sedge Point, at a location approximately 0.6 mile distant in a straight line from the shelters at Hook Point and about 1 mile from the Grapevine Point constructions. These new

shelters were removed twenty times, control areas were seined five times, and weed beds west of the shelters were seined nine times between July 25 and August 17, 1939. For the shelters at a 10-foot depth and the weed beds a 6 × 80-foot section of seine was fastened to the top of the middle section of the 140-foot seine used in all seining operations. At Sedge Point twelve large rock bass were tagged, of which four were retaken; fifteen small-mouthed bass were tagged, and seven of these were renetted; seven large-mouthed bass and six northern pike were taken from the weed beds along the drop-off just west of the shelter, four large-mouthed bass and one pike being recaptured in the same weed beds. None of the fish tagged at Sedge Point were seined again at any other location, nor were any of the fish marked at Grapevine Point caught at Sedge Point. Of the fish set free at East Point, only two rock bass were recovered at Sedge Point (one was taken a second time).

The weed beds along the drop-off where Big Shoal and the deep water of South Fishtail Bay meet (see Fig. 1) was chosen as another spot for the attempted recapture of some of the marked fish. This area lies roughly 0.5 mile in a straight line from the Grapevine Point shelters, about 1.2 miles from the Sedge Point seining grounds, and about 1.5 miles from the Hook Point installations. Four seine hauls with the 140-foot bag seine, to which the 6 × 80-foot section of seine had been fastened, did not take any marked fish, although a total of 549 of the species marked in Douglas Lake were caught.

In a further effort to secure marked fish fyke nets were set in several places, as specified below, between July 28 and August 17, 1939. Twelve small-mouthed bass caught in the fyke nets were tagged and let go. One of these bass, tagged and liberated about 200 feet east of the point of Big Shoal, was later recaptured in a fyke net about 0.2 mile farther east along the south drop-off of Big Shoal. A small-mouthed bass released at East Point was later found in a fyke net at Pine Point. Eight large rock bass and eleven large pumpkinseeds were tagged and set free at the fyke net settings, but none of these were retaken. No other marked fish were taken in the fyke nets.

### THE MOVEMENTS OF PLANTED SMALL-MOUTHED BASS IN DOUGLAS LAKE

On June 27 and July 13 and 24, 1939, a total of 271 small-mouthed bass of almost the legal size of 10 inches or larger were received from

Lake Michigan and released at the boat dock of the Biological Station, after they had been measured and weighed and scale samples had been taken. The shore from Grapevine Point to Pine Point was checked every morning for dead fish (8.5 per cent of the planted bass were found dead during the first nine days after planting). Posters were placed at the various resorts on the lake asking fishermen to report any tagged fish caught. Three per cent of the planted fish were retaken with nets and 6.3 per cent were caught and reported by fishermen between July 16 and September 1, 1939. The eight fish recovered with nets were all taken around the shelters at Grapevine Point. The seventeen small-mouths taken with hook and line were found in different parts of the lake. The place and number of recaptures are indicated by the circled figures on the map (Fig. 1). Two fish were hooked at the tip of Fairy Island, one in the bay near Bryant's, one at Stony Point, three at Pine Point, two along the south side of Big Shoal, one on the drop-off of the depression in South Fishtail Bay, approximately 0.4 mile east of the Biological Station, and seven in the weed beds on the drop-off at the Biological Station boathouse. The number of planted small-mouthed bass retaken is too small to justify very definite conclusions, but even this scant information seems to indicate that planted fish do more roaming than the native fish.

## DISCUSSION AND SUMMARY

The results of three summers' work at Douglas Lake quite definitely indicate that there is little movement of the native game fish from one part of the lake to another. Of all the fish marked at several locations in Douglas Lake and loosed at the point of capture none were retaken in distant parts of the lake. Recaptures were made only in the near vicinity of original capture and release.

Many game fish (4,557) were marked for three consecutive years at Grapevine Point but none were caught again at any other location. Seining operations were performed at several places located 0.5 of a mile to 1.5 miles in a straight line from Grapevine Point. A limited number of fish (forty) were tagged and freed at Sedge Point in 1939. All recoveries of these marked fish (sixteen) were made in the vicinity of Sedge Point.

Fyke net settings, the nearest of which was only about 800 feet from the Grapevine Point shelters on the south drop-off of Big Shoal,

did not take any marked fish from Grapevine Point. Weed-bed seinings along the southeast shore of South Fishtail Bay failed to bring in any marked fish.

In the work at Hook Point in 1938 and 1939 no marked fish were netted from any other place of release except a few which had been caught at Hook Point but liberated near East Point.

In 1938 and 1939 posters were placed around Douglas Lake asking fishermen to report the catching of any marked fish. Only two rock bass were reported, both of which were tagged in 1938 at Hook Point and released at East Point. One was caught at Pine Point in 1938, the other was secured at the shelters at Hook Point in July, 1939. The paucity of these returns may be explained in part by the fact that little fishing is done for rock bass, yellow perch, or pumpkinseeds in Douglas Lake.

This evidence brings out rather conclusively the fact that there is little movement of marked native fish in the eastern end of Douglas Lake when such fish are released at the point of first capture. They do not seem to migrate to new locations that offer similar habitats. One factor which may tend to inhibit such dissemination is the fact that Big Shoal almost separates North Fishtail Bay from South Fishtail Bay. The water over the greater part of Big Shoal is shallow (5 feet or less in depth) and almost devoid of cover; and the shoal is surrounded on three sides by deep water with rather steep drop-offs which may tend to retard fish from swimming across the wide, open shoal. The deep waters of the lake may also act as barriers to the free dispersion of fish.

Recaptures of the marked fish at Grapevine Point indicate a restricted movement even within a short distance along one shore. The evidence that fish wander little in Douglas Lake is in line with the fact that the fishing boats congregate in certain favored spots, which are known to the local guides who make a business of taking sportsmen to good fishing grounds. Some locations are known as bass fishing grounds, others as pike grounds. Indications are that fish congregate in these particular places year after year, since the guides use them every season and are very particular to get on the exact spot, for they claim that a difference of a few boat lengths will affect the fishing. Of course the abundance of fish on certain fishing grounds may be due more to the attractiveness of the habitat to the fish than to their restricted movements.

That the fish populations are localized in Douglas Lake is supported by observations made in 1921 by Dr. Carl L. Hubbs and Dr. Charles W. Creaser, who found evidence that the perch in the northwestern part of the lake are of the type occurring in Bessy Creek and Lancaster Lake, of which it is the outlet, rather than of the type occupying other parts of Douglas Lake (personal communication). Investigations by Dr. Frank E. Eggleton present further evidence that there are distinct perch populations in Douglas Lake (personal communication).

There is other evidence that Douglas Lake is not a simple unit. Limnological investigations (Welch, 1928, and Welch and Eggleton, 1932, 1935) disclose that the six major submerged depressions in the main basin of Douglas Lake act as independent lakes, each with its own physical, chemical, and biological characteristics.

The capture at Sedge Point, Grapevine Point, and Pine Point of fish that had been seined at Hook Point and returned to the water at East Point suggests that native fish which are taken from the place of capture and planted in another part of a lake do move from the point of release to other places in the lake.

Recoveries at numerous points in Douglas Lake of planted small-mouthed bass indicate that such fish wander more than native fish. The stocked bass seem to move around in the lake almost at random except that they apparently seek some shelter, since all recaptures were made either at the brush constructions or in weed beds.

University of Michigan

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# A LIMNOLOGICAL INVESTIGATION OF A PERMANENTLY STRATIFIED LAKE IN THE HURON MOUNTAIN REGION OF NORTHERN MICHIGAN *

LLOYD L. SMITH, JR.

DURING the progress of a fisheries investigation on the waters of the Huron Mountain Club in Marquette County, Michigan, observations were made on an uncommon type of lake. The physical and chemical conditions existing at all seasons of the year indicate permanent stratification, with an incomplete vernal and autumnal overturn. References in the literature to similar American bodies of water are few. Eggleton (1931) gave some evidence that Kirkville Green Lake in New York State may be stagnant or, at least, that permanent stagnation is possible. The physiography of its basin and certain characteristics in the distribution of its bottom fauna point to this conclusion. Welch (1935) mentioned small lakes which show physiographic characters indicating incomplete overturn. Ruttner (1931) discussed similar conditions in a tropical lake. Findenegg (1933, 1937) reported on lakes in central Europe that had incomplete overturn and permanent chemical stratification. Yoshimura (1937) investigated several lakes in Japan with permanent stagnation and lists a group of similar lakes in various parts of the world.

The author wishes to thank Messers Charles H. Hodges, Jr., and Wetmore Hodges, who have made the study possible by their financial support; the management and membership of the Huron Mountain Club for coöperation in the field work; Drs. Carl L. Hubbs and Paul S. Welch for their helpful suggestions and criticism of the manuscript; and Mr. J. P. Harris for assistance in laboratory examinations.

* This study was made as a part of a doctoral dissertation presented to the Department of Zoölogy of the University of Michigan.

PHYSIOGRAPHY

Canyon Lake is at the western end of an old range of pre-Cambrian granite hills lying adjacent to the southern shore of Lake Superior. The region was covered by the Wisconsin ice sheet, which scoured off the peaks and left rounded knobs that rise 1,000 feet above the present level of the Great Lakes. During the Algonquin stage of the last glaciation the waters impounded by the retreating ice completely covered the basin of Canyon Lake. With the subsequent recession of the waters this lake was isolated, and it now lies 233 feet above the present level of Lake Superior (Scott, 1921; Leverett, 1929).

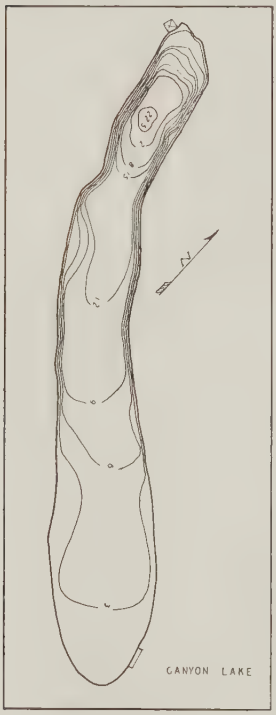


FIG. 1. A contour map of Canyon Lake in the Huron Mountains, Marquette County, Michigan. Contour lines are at 3-meter intervals

Geologically the lake belongs to a type infrequent in Michigan. According to Scott (1921), it is a fault lake lying in a depression created by vertical displacement of the rock strata and subsequently dammed with morainic material. The basin is long and narrow, and covers an area of slightly more than ten acres (Fig. 1). Sheer crystalline rock cliffs rise out of the water to a height of 50 feet and form most of the lateral margins. Near the southeast end these walls recede from the water and give place to a swamp which blocks the overflow from the lake. A maximum depth of 22.5 meters is attained near the north end of the lake. As Figure 1 indicates, the shoal area is limited to a small region at the shallow end. All other shores plunge off almost vertically into deep water.

The watershed is small and for the most part is composed of granite rock overlain with a thin glacial soil. Except on the bare rock surfaces the area supports a hemlock–hardwood forest. The

precipitous banks of the lake give foothold to a few white cedars and pines. Bottom soils are composed more or less uniformly of a grayish green pulpy peat containing much organic detritus from the surrounding forest floor. Submerged aquatic vegetation is sparse and restricted to the first three meters of shoal water at the shallow end.

The high banks and the surrounding hills almost eliminate wave action during the open-water season. An ice sheet formed in late November and remaining until early May closes the lake during the winter months. The ice reaches a thickness of 18 to 24 inches and is covered with 1 to 3 feet of snow.

## METHODS AND PROCEDURE

Ten series of observations over a period of two years were taken on Canyon Lake to secure data for all the limnological seasons. A maximum-minimum thermometer was used for all temperature determinations. Dissolved oxygen, total alkalinity, free $CO_2$, and pH determinations were made in accordance with the procedure outlined in *Standard Methods for the Examination of Water and Sewage* (1933). Ten-liter samples of water from various depths were secured with a two-liter Kemmerer-Juday water bottle and were poured through a no. 25 bolting-silk plankton net to remove free-floating forms. The concentrated plankton was preserved in a 10 per cent formalin solution. Samples of the bottom soil, taken with a 6 × 6-inch Eckman dredge, were mixed with clean water and sieved through no. 40 brass screens, and the concentrated bottom organisms and detritus were preserved in a 10 per cent formalin solution. The minnow population was sampled with a net 6 feet in diameter operated from a boat. The larger species of fish were sampled with experimental gill nets, which ranged from $\frac{3}{4}$- to 2-inch bar mesh. Inaccessibility of the lake during the winter months limited the observations at that season to chemical analyses.

## PHYSICOCHEMICAL CHARACTERISTICS

### *1. Temperature*

Surface temperatures in Canyon Lake vary directly with the air temperature and follow it more closely than they do in the larger adjacent bodies of water. As will be pointed out later, the shallow

depth of the epilimnion accounts for this rapid response. Below 8 meters the temperature remains constant at 4.4° C. at all seasons. A thermocline, which may be formed as early as the middle of May (Fig. 2), persists until late September or early October. Its upper limits lie between 2 and 3 meters; the lower ones, between 6 and 8 meters. It is formed near the surface as a relatively thin layer (Fig. 2), but with the advance of summer it gradually increases in thickness by depression of the lower limit (Fig. 3). During the

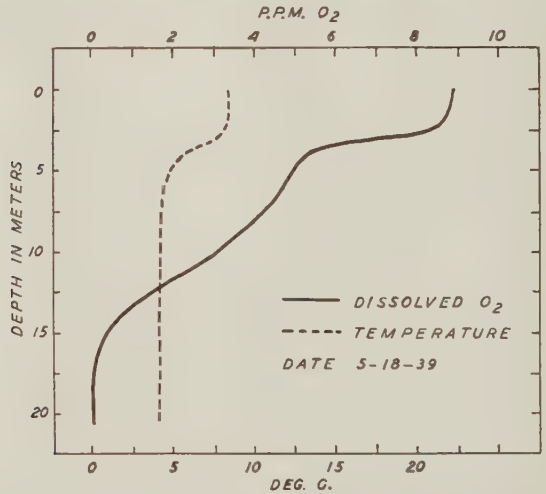


FIG. 2. Temperature and dissolved oxygen concentration from surface to a depth of 22 meters on May 18, 1939

hottest part of the year the thermocline, strictly defined, starts almost at the surface (Fig. 4). The rapid decline of temperature near the surface virtually eliminates the epilimnion and prevents circulation of the surface water. With the cooling of the surface waters in the fall the thermocline becomes less distinct and finally disappears, which allows some degree of circulation in the upper waters (Figs. 5–6). The clarity of the water and the restricted area of the basin would normally cause higher surface temperatures and a lower position of the thermocline, but the abrupt and closely approximated walls of the lake shade the waters except for a short time during midday.

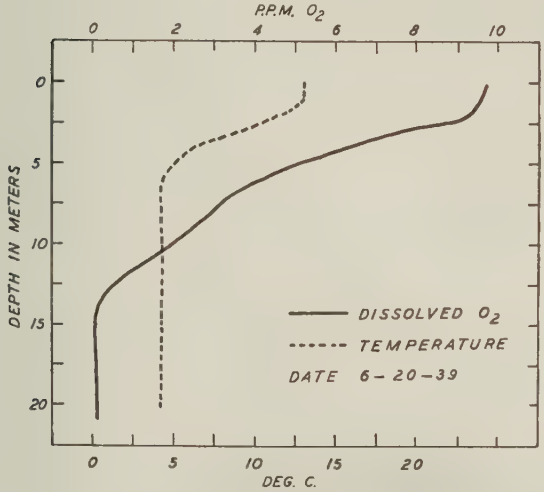


FIG. 3. Temperature and dissolved oxygen concentration from surface to a depth of 22 meters on June 20, 1939

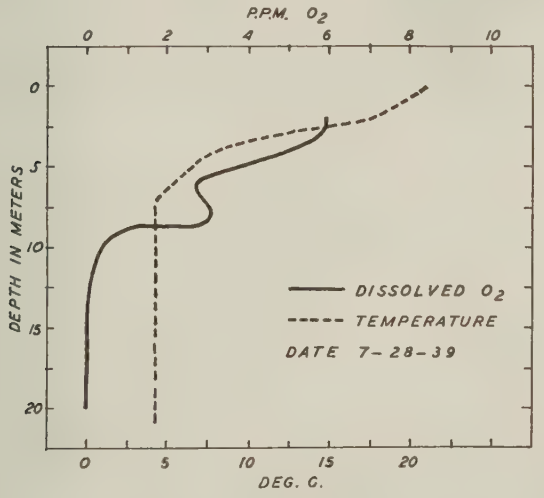


FIG. 4. Temperature and dissolved oxygen concentration from surface to a depth of 22 meters on July 28, 1939. The irregularities near the thermocline are correlated with the density of phytoplankton

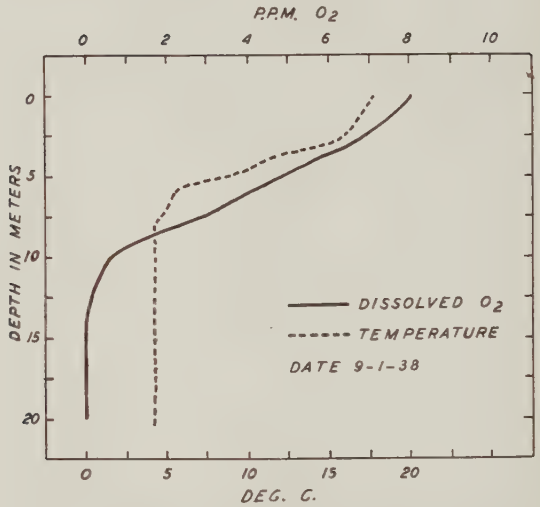


FIG. 5. Temperature and dissolved oxygen concentration from surface to a depth of 22 meters on September 1, 1938

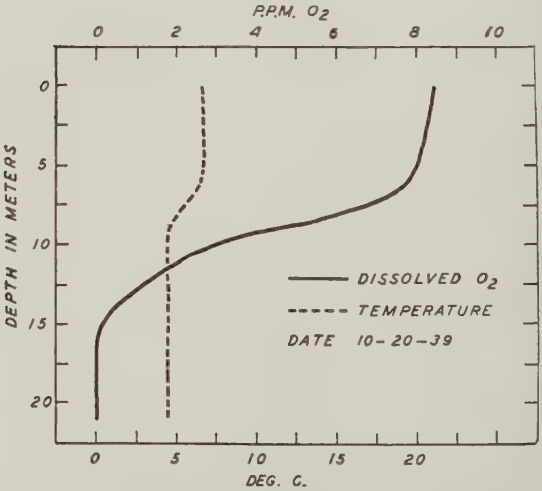


FIG. 6. Temperature and dissolved oxygen concentration from surface to a depth of 22 meters on October 20, 1939

Aside from the shallow position of the thermocline there is nothing particularly significant about the temperature phenomena. The thermal characteristics, however, are the only physicochemical factors which vary normally with the change of the seasons. Unusual chemical conditions exist throughout the year and give strong indication of permanent water stratification.

### *2. Dissolved Oxygen*

Dissolved oxygen is not present below 16 meters at any season, and during the summer the last traces are found at 12 meters (Fig. 4). In May oxygen occurs to a depth of 16 meters (Fig. 2). As the season advances it is gradually eliminated from the lower strata until in late July none is found below 12 meters. At this time the curve of oxygen distribution is very irregular in the lower levels of the thermocline because of the operation of biotic factors. By September 1 these irregularities have straightened out, and there is a gradual decrease in concentration from the surface to the 12-meter depth (Fig. 5). By late October the overturn of the upper waters has begun, and oxygen is again present at 15 meters (Fig. 6). It will be noted that the overturn is incomplete and that the distribution of oxygen is not uniform, but shows a gradual decrease from the surface to the point of disappearance. In late winter oxygen in the upper strata is reduced to 3.9 parts per million, whereas the lower strata remain anaërobic (Fig. 7).

### *3. Total Alkalinity and Free Carbon Dioxide*

Even more marked than the distribution of dissolved oxygen is the extreme variation from surface to bottom of the total alkalinity and the free $CO_2$. The alkalinity, expressed as parts per million of calcium carbonate, ranges from a minimum of 12 at the surface to a maximum of 270 at the bottom. Down to a depth of 15 meters the concentration increases to approximately three times the value at the surface, and then jumps rapidly to twenty times the surface value at the greatest depth. Minor variations occur during different seasons (Figs. 8–12), but the shape of the distribution curves remains essentially the same. During late July conditions in the thermocline cause irregularities similar to those noted for oxygen.

The content of free $CO_2$ varies from 1 part per million at the surface to a maximum of 232 parts per million at the 21-meter level.

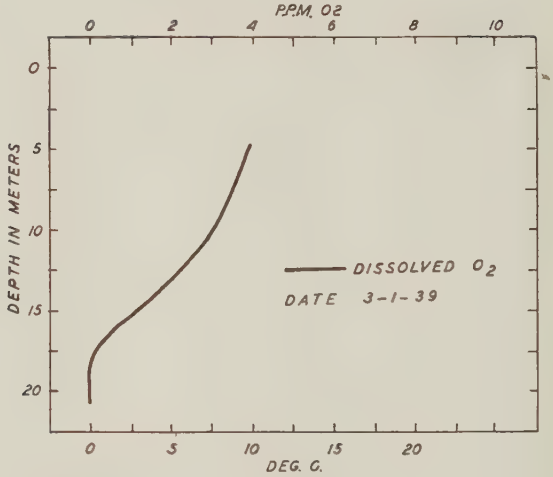


FIG. 7. Dissolved oxygen concentration on March 1, 1939. Eighteen inches of ice covered the lake at the time of this observation

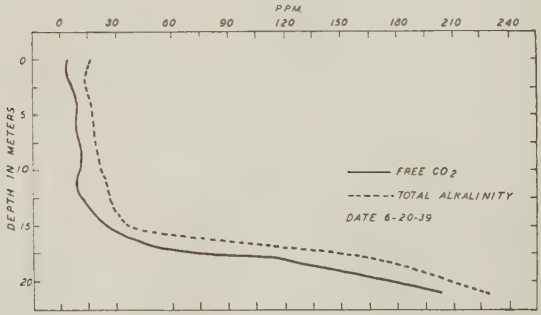


FIG. 8. Total alkalinity, expressed as parts per million of calcium carbonate, and free $CO_2$ concentrations from surface to a depth of 22 meters on June 20, 1939

The gradual increase in concentration to a depth of 15 meters and the rapid increase below that point are as notable as for the total alkalinity. The curves for free $CO_2$ remain relatively constant at all seasons and follow the general shape of the alkalinity curves. Winter observations were not made at the maximum depths for either $CO_2$ or

total alkalinity, but the conditions down to 18 meters were very similar to those in late October and May.

When brought to the surface from 18 meters and below the water samples effervesced because the release of pressure and increase in

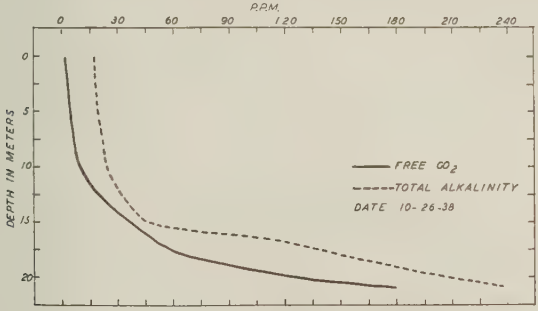


Fig. 9. Total alkalinity, expressed as parts per million of calcium carbonate, and free $CO_2$ concentrations from surface to a depth of 22 meters on October 26, 1938

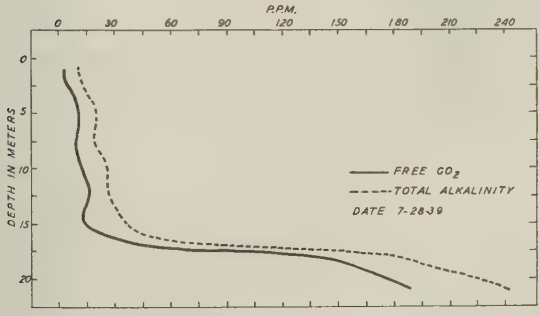


Fig. 10. Total alkalinity, expressed as parts per million of calcium carbonate, and free $CO_2$ concentrations from surface to a depth of 22 meters on July 28, 1939

temperature liberated a gas held in solution. No field equipment for the analysis of this gas was available. Since a flame test showed no methane and since no odor of hydrogen sulfide was present, the gas was assumed to be $CO_2$. The high concentration of $CO_2$ in the water and the precipitation of ferric salts in sample bottles after a period of standing lend support to this suggestion.

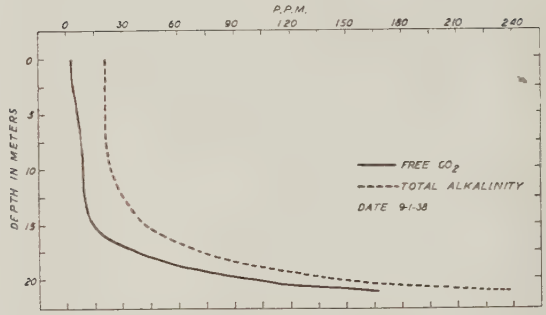


FIG. 11. Total alkalinity, expressed as parts per million of calcium carbonate, and free $CO_2$ concentrations from surface to a depth of 22 meters on September 1, 1938

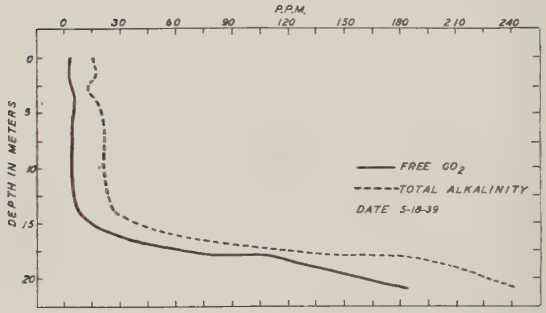


FIG. 12. Total alkalinity, expressed as parts per million of calcium carbonate, and free $CO_2$ concentrations from surface to a depth of 22 meters on May 18, 1939

### *4. Hydrogen-Ion Concentration*

The pH ranges from 7.0 at the surface to 6.0 at the bottom, with no very significant seasonal changes. The highest hydrogen-ion concentration occurs during the winter and the lowest during the spring and fall (Fig. 13). During midsummer there is a sharp drop of pH in the region of thermal stratification. The relatively high pH associated with the high concentration of dissolved $CO_2$ is probably to be explained by the action of natural buffers.

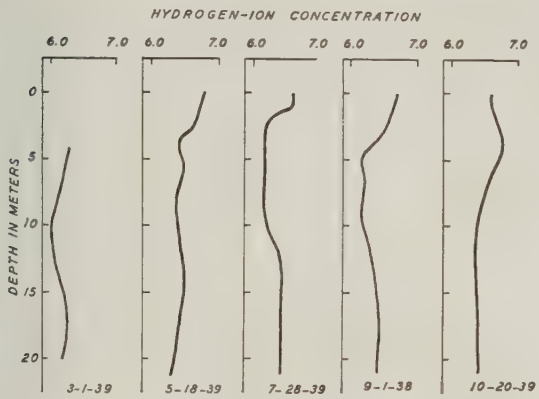


FIG. 13. Hydrogen-ion concentration at different seasons. The depression in July, 1939, is correlated with the activity of the plankton at that level

## BIOLOGICAL CHARACTERISTICS

As would be expected from the alkalinity of the surface waters, the biological productivity of the lake is low, with few species and a light concentration of individuals. The fish population consists of the lake chub, *Couesius plumbeus*, and the speckled trout, *Salvelinus fontinalis*. Hubbs (1929) lists the chub as the only native species and ascribes the absence of other species to the early isolation of the lake in postglacial time.

There are not many benthic organisms, and the number of species is small. The most prominent species are *Hyalella knickerbockeri* in the shoal waters and *Corethra sp.* and several species of Chironominae in deeper water. At all seasons of the year the bottom soils below 16 meters are free of macroscopic organisms. In midsummer none are found below 10 meters. There is a marked irregularity of distribution, emphasized by two concentration zones. During late July the upper zone lies between 1 and 3 meters and the lower between 6 and 7 meters (Fig. 14). At this time the lower concentration is dominated by Chironominae larvae, but as fall approaches it begins to move down, until in late October it lies at 8 meters, with *Corethra sp.* and Chironominae dominating the population. Along with this downward shift is the appearance of *Corethra* at 15 meters

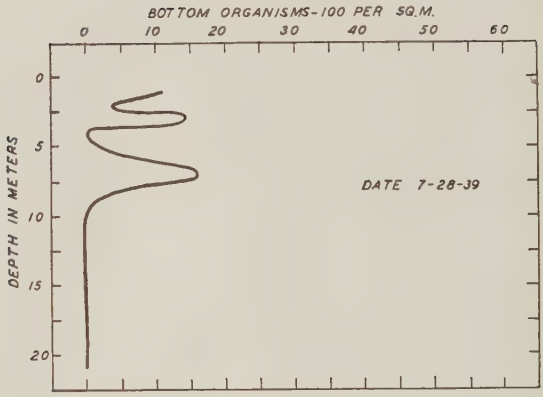


Fig. 14. Distribution of bottom organisms on July 28, 1939. The concentration zone, composed of *Corethra* and Chironominae, lies at a depth of between 6 and 7 meters

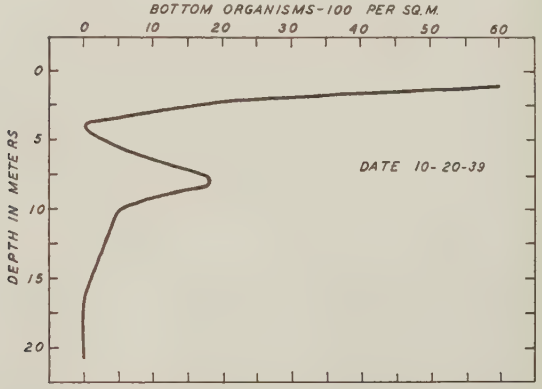


Fig. 15. Distribution of bottom organisms on October 20, 1939. The concentration zone lies at a depth of 8 meters, and organisms have penetrated to 15 meters

(Fig. 15). Coincident with changes in the location of concentrations are the increase of oxygen in the upper strata and the penetration of traces to 16 meters (Fig. 6). The concentration zone during midsummer lies in the lower stratum of the thermocline, or just below it. With the disappearance of thermal stratification it moves to

greater depths. Eggleton (1931) has shown that this phenomenon is more or less typical of eutrophic lakes.

In May, shortly after the ice goes out, a concentration zone composed entirely of *Corethra* lies at 14 meters (Fig. 16). As the season progresses the zone moves up to the summer level, and all organisms disappear from the lower strata. The tendency toward downward movement in the fall and the low position of the zone just after the spring break-up suggest that the greatest number of organisms occur at the lower level of available oxygen during the

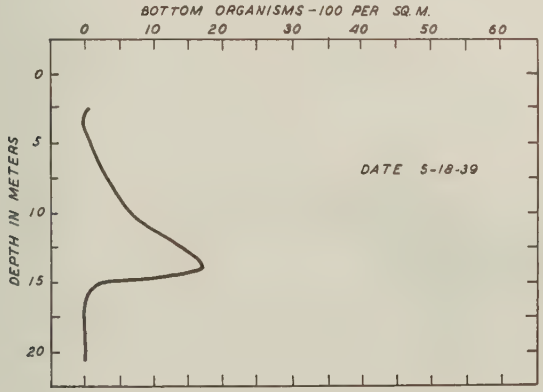


Fig. 16. Distribution of bottom organisms on May 18, 1939. The concentration zone, composed of *Corethra*, lies at a depth of 14 meters

winter months. Eggleton (1931) showed this to be the usual condition in the lakes he studied. A peculiarity which occurs at all seasons is the complete absence of organisms at the 4-meter level. Since there is no apparent temperature or chemical condition correlated with this barren area, the cause may be some peculiarity of the bottom soil.

In general, the distribution of the bottom organisms seems to depend on the availability of oxygen. The permanent depletion of oxygen in the lower levels is correlated with the absence of bottom forms, and the seasonal disappearance of life from the middle zone is likewise coincident with oxygen removal.

In this lake there is nothing remarkable about the species of

plankton or their distribution, and they tend to bear out the assumptions derived from the other limnological data. The number of both species and individuals is relatively low, and nothing approaching a typical plankton surge has been observed. The zooplankton is dominated by the Rotifera; the phytoplankton, by *Tabellaria sp.*, *Dinobryon sertularia*, and *Ceratium hirundinella*. The scarcity of blue-green algae and the small number of phytoplankters may be due in part to the low temperature of the water and the few hours of sunlight which the rock walls permit to strike the water.

The waters of the stagnation zone are dominated at all seasons by *Keratella cochlearis*, *Ceratium hirundinella*, and *Tabellaria sp.* The only cladoceran observed in these strata was *Bosmina longispina*. At one period in late summer a considerable increase in *Dinobryon* occurred and was noticeable as deep as 15 meters. Since there were no apparent physical or chemical changes at this level and since the increase was not reflected by other species, the abundance of this particular form may have been caused by the sinking of individuals from overlying layers. At 20 meters the plankton consisted entirely of *Keratella cochlearis*, *Ceratium hirundinella*, *Tabellaria sp.*, and *Diatoma sp.* The population is marked by the absence of deep-water forms which are commonly found in cold, soft lakes without stagnation.

From Table I, which shows the total number of plankton forms and the total number of zooplankters per liter at various depths and seasons, it will be noted that the decrease in numbers from surface to bottom is largely due to the decrease in phytoplankton. The irregularity of oxygen distribution in late July previously pointed out is correlated with the concentration of algal forms in the thermocline.

The following list of plankton species, although by no means complete, contains the conspicuous and numerically dominant forms.

Rotifera: *Polyarthra platyptera, Triarthra longiseta, Pedetes saltator, Keratella cochlearis, Keratella quadrata, Notholca longispina, Rattulus sp.*
Cladocera: *Bosmina longispina, Daphnia longispina.*
Dinoflagellida: *Ceratium hirundinella.*
Phaeophyceae: *Dinobryon sertularia.*
Diatomaceae: *Tabellaria sp., Diatoma sp., Fragillaria sp.*

In some of the surface samples *Microcystis, Diaptomus sp., Cyclops sp.*, and *Ceriodaphnia sp.* were occasionally present. A few

TABLE I

TOTAL NUMBER OF PLANKTON INDIVIDUALS AND TOTAL NUMBER OF ZOOPLANKTERS PER LITER

| Depth in meters | July 28, 1938 | | Sept. 1, 1938 | | Oct. 25, 1938 | | May 18, 1939 | | Oct. 21, 1939 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Total plank. | Zoo-plank. | Total plank. | Zoo-plank. | Total plank. | Zoo-plank. | Total plank. | Zoo-plank. | Total plank. | Zoo-plank. |
| 0 | 58,936 | 21,425 | 9,918 | 178 | 1,115 | 1,052 | 91 | 28 | 6,836 | 4,510 |
| 2 | 1,977 | 279 | ... | ... | ... | ... | 210 | 60 | ... | ... |
| 3 | ... | ... | 4,417 | 139 | 293 | 146 | 35 | 28 | ... | ... |
| 4 | ... | ... | 1,281 | 223 | ... | ... | 158 | 52 | ... | ... |
| 5 | ... | ... | ... | ... | 471 | 339 | ... | ... | ... | ... |
| 6 | 448 | 122 | ... | ... | ... | ... | 36 | 28 | 315 | 289 |
| 7 | ... | ... | 244 | 110 | ... | ... | ... | ... | ... | ... |
| 8 | 1,236 | 710 | ... | ... | ... | ... | ... | ... | ... | ... |
| 10 | 1,000 | 1,000 | ... | ... | 46 | 46 | 77 | 14 | 81 | 44 |
| 14 | ... | ... | ... | ... | ... | ... | 14 | 12 | ... | ... |
| 15 | 206 | 123 | 1,426 | 106 | 50 | 29 | ... | ... | 40 | 21 |
| 18 | ... | ... | ... | ... | ... | ... | 19 | 15 | ... | ... |
| 20 | 42 | 39 | 83 | 35 | ... | ... | ... | ... | 52 | 20 |

other species of phytoplankton and rotifers formed inconspicuous components of the population.

From consideration of the physical, chemical, and biological data it appears that Canyon Lake has a more or less normal circulation zone extending down to a depth of 15 meters and a permanent stagnation zone below that level. This permanent stratification limits the distribution of macroscopic bottom organisms and strictly aërobic plankton forms to the upper strata of water, and makes the lower strata practically barren.

SUMMARY

1. The deep strata of the lake remain constantly at 4.4° C.

2. There is a sharp thermal stratification, with the upper limits of the thermocline lying between 2 and 3 meters.

3. The lake contains no trace of dissolved oxygen below 16 meters at any time of the year.

4. The total alkalinity and free $CO_2$ show a great variation from surface to bottom which does not change significantly during the year.

5. The lake is a soft-water type in its upper strata and a modified hard-water type in the lower strata.

6. No macroscopic bottom organisms occur below 16 meters.

7. Above 16 meters the seasonal changes in the benthos are similar to those in a eutrophic lake.

8. The plankton species dominant in the stagnation zone are those which frequently occur in anaërobic conditions.

9. The lake has the appearance of two separate bodies of water, one superimposed upon the other: the upper active and the lower stagnant.

10. Permanent stratification and the resulting physical, chemical, and biological phenomena are caused by the low temperature of the bottom waters, the precipitous banks, and the lack of wind agitation.

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# ZOOGEOGRAPHY OF NAIADES IN THE GRAND AND MUSKEGON RIVERS OF MICHIGAN AS RELATED TO GLACIAL HISTORY

HENRY VAN DER SCHALIE

ALTHOUGH Michigan lies wholly within the St. Lawrence drainage basin, the fresh-water mussels of the rivers of southern Michigan are entirely Mississippian. Furthermore, the areas drained by the highly productive streams in and south of the valleys of the Saginaw and Grand rivers, which were formerly connected, are very similar in their soil composition, topography, and climate. There are no great natural barriers to distribution over land, yet there are marked faunal differences in the distribution of the species of naiades in the various streams of this region. These differences have not been explained satisfactorily by any of the observable variations in the present conditions of the drainage basin. In the past practically all of them have been well explained as due to some event in glacial history, and this continues to be the best way of accounting for them.

There were two major avenues by way of which Mississippian naiades entered southern Michigan. These pathways have been discussed by Bryant Walker (1898, 1913). One entrance into southern Michigan, elaborated upon by Ortmann (1924) and van der Schalie (1938a), was through the Maumee River outlet to the Wabash and Mississippi rivers. This confluence permitted the thirty species of mussels now occupying Lake Erie and its tributaries to enter southeastern Michigan. The other avenue, the one by which naiades entered southwestern Michigan, was through the Des Plaines River and the Chicago outlet to the Mississippi River. It is with the latter that we are primarily concerned at present. Some details of the glacial history as it relates to the events attending the receding ice lobes of the last or Wisconsin stage of glaciation in the western portion of Michigan will be discussed later, in explaining the distribution of mussels in the Grand and Muskegon rivers.

A study of the naiades of the Muskegon and Grand rivers suggests

TABLE I

FAUNAL COMPARISON OF THE NAIADES IN THE GRAND AND MUSKEGON RIVERS

| *Species* | *Grand River* | *Muskegon River* |
|---|---|---|
| Quadrula pustulosa | + | − |
| Quadrula quadrula * | +† | − |
| Cyclonaias tuberculata | + | − |
| Amblema costata | + | − |
| Amblema peruviana | + | − |
| Fusconaia flava | + | + |
| Pleurobema cord. coccineum | + | + |
| Elliptio dilatatus | + | + |
| Strophitus rugosus | + | + |
| Anodonta grandis | + | + |
| Anodonta imbecillis | + | − |
| Anodontoides ferussacianus | + | + |
| Lasmigona compressa | + | + |
| Lasmigona complanata | +† | +† |
| Lasmigona costata | + | + |
| Alasmidonta calceolus | + | + |
| Alasmidonta marginata ‡ | + | + |
| Obliquaria reflexa | +† | +† |
| Proptera alata | +† | +† |
| Leptodea fragilis | +† | +† |
| Obovaria olivaria * | +† | − |
| Actinonaias carinata ‡ | + | + |
| Actinonaias ellipsiformis ‡ | + | + |
| Micromya iris | + | + |
| Ligumia recta latissima | + | + |
| Lampsilis siliquoidea | + | + |
| Lampsilis ventricosa | + | + |
| Truncilla donaciformis | +† | − |
| Truncilla truncata * | +† | − |
| Dysnomia triquetra | − | +† |
| Total | 29 | 21 |

* Reported by R. E. Coker (1921).
† Species entering mouth of river from Lake Michigan.
‡ Species with discontinuous distribution.

that the Muskegon was formerly a tributary of the Grand River. A comparison of the species inhabiting the two rivers is presented in Table I. The Muskegon River is shown to harbor only twenty-one of the twenty-nine species found in the Grand River. Five of them, marked by daggers, are known to be common to the Great Lakes (Goodrich and van der Schalie, 1932), and they probably entered the lower portion of the Muskegon River from Lake Michigan (Table II).

# TABLE II

## Distribution of Naiades in the Muskegon River

| Species | Tributaries | | | | | | | | | Main River | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1. Wolf Cr., 10 mi. s. of Houghton | 10. Dishwash Cr., at Marion | 11. Middle Branch R., 2 mi. s. of Marion | 19. E. Br. Little Muskegon R., Mecosta | 21. Tamarack Cr., at Howard City | 9. W. Br. Clam R., 5 mi. e. of Marion | 8. Clam R., 7 mi. e. of Marion | 20. Little Muskegon R., sw. of Altona | 18. Ryan Cr., 2 mi. se. of Big Rapids | 2. Between Higgins and Houghton lakes | 3. 3 mi. below Houghton Lake | 4. 7 mi. w. of Houghton Lake | 5. ¼ mi. w. of Leota | 7. Just w. of Temple | 12. 11 mi. s. of Marion | 13. 1 mi. ne. of Evart | 14. Below Hersey | 15. Above Paris | 16. At Big Rapids | 17. Below Roger's Dam | 22. Below Croton Dam | 23. 3 mi. below Newaygo | 24. At Bridgeton | 25. 10 mi. ne. of Muskegon |
| Elliptio dilatatus | .. | .. | .. | .. | .. | .. | .. | 13 | 30 | 33 | 71 | .. | 3 | 7 | 20 | 75 | 49 | 55 | .. | 3 | 2 | 8 | .. | 1 |
| Fusconaia flava | .. | .. | .. | .. | .. | .. | .. | 1 | 20 | 2 | 2 | 1 | 10 | 2 | 5 | 10 | 6 | 9 | .. | 1 | 1 | 27 | 1 | .. |
| Pleurobema cord. coccineum | .. | .. | .. | .. | .. | .. | .. | .. | 6 | .. | .. | .. | 1 | .. | .. | .. | 3 | .. | .. | 1 | 1 | 1 | 1 | .. |
| Strophitus rugosus | .. | .. | .. | .. | .. | .. | 5 | 4 | 5 | 1 | 3 | .. | 1 | 1 | 4 | 2 | 17 | 14 | 1 | 5 | 47 | 21 | 1 | 1 |
| Lasmigona costata | .. | .. | .. | .. | 1 | .. | .. | 4 | 1 | .. | 1 | .. | .. | .. | 5 | 12 | 14 | 3 | 2 | 2 | 1 | .. | .. | .. |
| Lasmigona compressa | .. | 1 | .. | 4 | 1 | 1 | 3 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Anodonta grandis | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 38 | .. | .. | .. | .. | .. | .. | .. | .. | 1 | 1 | 2 | 1 | .. |
| Alasmidonta calceolus | 2 | .. | 1 | .. | 3 | 4 | 3 | .. | 6 | .. | .. | .. | .. | .. | 2 | 1 | 1 | .. | .. | .. | .. | .. | .. | .. |
| Alasmidonta marginata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 2 | .. | .. | 5 | 4 | .. | 2 | .. |
| Anodontoides ferussacianus | .. | .. | 6 | 2 | .. | 16 | 38 | 1 | 1 | .. | .. | .. | .. | 2 | .. | .. | .. | .. | .. | .. | 2 | .. | .. | .. |
| Micromya iris | .. | .. | .. | .. | .. | .. | .. | .. | 4 | 30 | 5 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Lampsilis siliquoidea | 18 | 1 | 11 | .. | .. | 6 | 20 | 2 | 3 | .. | 46 | .. | 77 | 45 | 31 | 57 | 25 | 25 | 17 | 3 | 15 | 5 | .. | .. |
| Lampsilis ventricosa | .. | .. | .. | .. | .. | .. | .. | .. | .. | 23 | 3 | .. | .. | .. | 6 | .. | .. | 1 | .. | 3 | 20 | 3 | .. | .. |
| Ligumia recta latissima | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 1 | .. | .. | 1 | 25 | 29 | 32 | 3 | .. | 7 | 4 | 2 | .. | .. |
| Actinonaias carinata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 16 | 8 | 6 | 13 | 2 | 5 | 4 | 31 | 5 | 1 |
| Dysnomia triquetra | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 6 | .. | .. |
| Proptera alata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 1 | .. |
| Total no. of species | 2 | 2 | 3 | 2 | 3 | 4 | 5 | 6 | 9 | 5 | 9 | 1 | 5 | 6 | 9 | 8 | 10 | 8 | 4 | 11 | 12 | 10 | 7 | 3 |

The remaining sixteen are primarily a medium-sized river assemblage. Two of them, *Actinonaias carinata* and *Alasmidonta marginata*, are normally inhabitants of rivers and have never been reported from lakes. Consequently, in the Muskegon River they have *discontinuous* distribution. Both are common in the Grand River, where also they are discontinuous in distribution. These data had led to the belief that at one time in its drainage history the Muskegon River was a tributary of the Grand River. This view is substantiated by the glacial history of these streams, since it is known that the Muskegon River was at one time connected with the Grand River through the valley of the present Rouge (Rogue [1]) River.

The course of the Muskegon River as it flowed through the valley of the Rouge River (north of Grand Rapids) was traced by Leverett and Taylor (1915, p. 227): "At one time during the recession of the ice border the Muskegon, which received drainage from the Lake Michigan lobe on the west and the Saginaw Bay lobe on the east, ran southward past Rice Lake in southeastern Newaygo County, its altitude near Rice Lake being 800 feet above sea level. It followed Rouge River to its junction with Grand River near Grand Rapids and probably continued southward (for a time at least), leaving the Grand River near Grand Rapids."

At the meeting of the Michigan Academy in 1902 Dr. Leverett discussed this drainage course in detail, but his address was never published. He assures me, however, that the entire geomorphology of the present Rouge River valley — its extensive width, the distribution of the sands and gravels, the swamp that now exists at the head of this valley, the shape of Rice Lake, and the present position of the Saginaw and Lake Michigan moraines, which indicates that ice once plugged the mouth of the Muskegon River — points to a former connection between the Muskegon and the Grand rivers.

There is considerable skepticism among zoologists, geologists, and physiographers regarding the value of distributional studies of naiades in tracing stream confluence. One prominent geomorphologist is convinced that the distribution patterns of mussels can be best explained on the assumption of a transfer of these animals by passive agents, such as birds and mammals. A well-known conchologist has expressed himself as in agreement with the contentions of

[1] Two spellings, "Rouge" and "Rogue," appear on maps. Old residents of the region drained by this river use "Rogue River."

the physiographer. I have pointed out earlier (van der Schalie, 1939) that the evidence that birds and mammals are the agents responsible for the distributional features observed among naiades in nature is meager and wholly inadequate. Yet in spite of the imposing array of *factual* data in opposition to their theory the protagonists of passive migration of mussels by agents other than fishes are obviously still unconvinced. This attitude is unfortunate, since their interpretation undoubtedly hinders progress in the utilization of the sound evidence that the distribution of naiades can give in tracing drainage lines of the past.

A common mussel of southwestern Michigan is *Actinonaias ellipsiformis*. It was found at many stations in the Grand River (Table III). On the other hand, *Lampsilis fasciola* is well represented in the three main rivers of southeastern Michigan (van der Schalie, 1938a, p. 14). Both species inhabit rivers, are associated ecologically with streams of medium size, and usually are not found in the Great Lakes. Hence both are at present *discontinuous* in their respective ranges. The distribution pattern of each is shown graphically in Figure 1. If naiades are distributed by birds and mammals, it would be interesting to have an explanation of the reason why neither of these species, *ellipsiformis* and *fasciola*, has been carried across the east–west drainage divide, particularly since the tributary streams of the two major drainage systems so closely approximate each other. Dr. Wilbert B. Hinsdale (1931, p. 6), in discussing famous portages in Michigan, points out that only a low divide separates the Raisin River from both the Kalamazoo and the Grand rivers. In regard to the divide between the Huron and the Grand rivers he says: "Mr. N. F. Wing, of Grass Lake, states that forty years ago he actually paddled across the divide between Portage River and Otter Creek, through the lowlands and swamps, without having to get out of his canoe. During high water the Indians certainly could have done the same thing; the only obstructions from Lake Erie to Lake Michigan would have been rapids and fallen timber." Nevertheless, owing to restrictions attending the ecology and distribution of naiades these species have not crossed this divide since glacial time. Certainly if birds and mammals are a factor in mussel distribution, such a restriction would not be likely to persist.

When the distribution of the species mentioned above is considered in relation to the late glacial history of the drainages inhabited by

TABLE

DISTRIBUTION OF NAIADES

| SPECIES | TRIBUTARIES | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 76. Rush Cr., 2 mi. e. of Hudsonville | | | 83. Cedar Cr., 4 mi. n. of Rockford | | | 82. Rouge R., 2 mi. n. of Rockford | | | 11. Red Cedar R., 1 mi. s. of Fowlerville | | | 12. W. Br., 4 mi. w. of Fowlerville | | | 13. 3 mi. e. of Webberville | | | 14. 2 mi. nw. of Webberville | | | 15. ¾ mi. w. of Williamston | | |
| Quadrula pustulosa | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Cyclonaias tuberculata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Amblema costata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Amblema peruviana | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Fusconaia flava | .. | .. | .. | .. | .. | .. | .. | .. | .. | 13 | .. | 1 | 19 | 4 | 15 | 2 | 8 | 18 | 12 | 7 | 1 | .. | 9 | .. | .. |
| Pleurobema cord. coccineum | .. | .. | .. | 5 | .. | .. | .. | .. | .. | .. | .. | .. | 17 | 1 | 14 | .. | 1 | 13 | 11 | .. | .. | .. | 4 | .. | .. |
| Elliptio dilatatus | .. | .. | .. | 7 | .. | .. | .. | .. | .. | .. | .. | .. | 1 | 7 | 63 | 7 | .. | 2 | 43 | 53 | .. | 2 | .. | 6 | 3 |
| Strophitus rugosus | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 22 | 6 | 23 | 1 | 3 | 2 | 9 | 20 | .. | 4 | 14 | 1 | 7 |
| Anodonta grandis | .. | .. | .. | .. | .. | .. | 2 | 5 | .. | .. | .. | .. | 1 | 1 | .. | .. | 4 | 2 | .. | .. | .. | .. | 1 | 1 | .. |
| Anodonta imbecillis | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Anodontoides ferussacianus | 5 | 1 | .. | 8 | 52 | .. | 1 | 4 | 2 | 2 | 4 | 3 | 3 | 1 | .. | 2 | .. | .. | .. | 5 | 15 | .. | .. | .. | .. |
| Lasmigona compressa | 1 | 3 | .. | 1 | 7 | .. | 1 | 1 | .. | 3 | 1 | .. | 1 | .. | .. | .. | 5 | .. | .. | .. | 1 | .. | .. | .. | .. |
| Lasmigona complanata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Lasmigona costata | .. | .. | .. | .. | .. | .. | .. | .. | .. | 2 | 1 | 1 | 1 | .. | .. | .. | 1 | .. | .. | .. | 3 | .. | .. | .. | .. |
| Alasmidonta calceolus | .. | 6 | 2 | 3 | 24 | 1 | 3 | 15 | 8 | 21 | 38 | 2 | 29 | 6 | 15 | .. | .. | .. | 1 | 1 | .. | .. | .. | .. | .. |
| Alasmidonta marginata | .. | .. | .. | .. | .. | .. | .. | .. | 1 | 1 | .. | .. | .. | .. | 9 | .. | .. | .. | .. | 2 | 7 | .. | .. | .. | .. |
| Proptera alata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Leptodea fragilis | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Actinonaias carinata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Actinonaias ellipsiformis | .. | .. | .. | 5 | .. | .. | .. | .. | 42 | 90 | 124 | 38 | .. | 25 | 37 | 66 | 1 | 10 | 5 | 13 | .. | .. | .. | .. | .. |
| Micromya iris | .. | .. | .. | .. | .. | .. | .. | .. | 1 | 23 | 151 | 150 | 1 | 16 | 14 | 22 | 23 | .. | 2 | 11 | 1 | .. | .. | .. | .. |
| Ligumia recta latissima | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 6 |
| Lampsilis siliquoidea | .. | .. | .. | .. | .. | .. | 40 | 1 | 7 | 19 | 1 | 4 | 33 | 26 | 12 | 1 | .. | .. | .. | 1 | .. | .. | .. | .. | .. |
| Lampsilis ventricosa | .. | .. | .. | .. | .. | .. | .. | .. | .. | 15 | 6 | 9 | .. | .. | 1 | 4 | 2 | 1 | 2 | 2 | 3 | 5 | .. | .. | .. |
| Truncilla donaciformis | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Total no. of species | 2 | 3 | 4 | 4 | 6 | 2 | 7 | 13 | 13 | 13 | 9 | 8 | 12 | 10 | 8 | 4 | 7 | 9 | 9 | 8 | | | | | |

| SPECIES | TRIBUTARIES | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 16. 5 mi. w. of Williamston | | | 17. Below Okemos | | | 18. Lookingglass R., 3 mi. n. of Perry | | | 19. 9 mi. w. of Laingsburg | | | 20. Near DeWitt | | | 22. 5 mi. w. of Lansing | | | 23. 2 mi. ne. of Eagle | | | 49. Wabasis Creek | | |
| Quadrula pustulosa | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Cyclonaias tuberculata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Amblema costata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Amblema peruviana | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Fusconaia flava | 4 | 15 | 2 | 8 | 18 | 12 | 7 | 1 | .. | 9 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Pleurobema cord. coccineum | 1 | 14 | .. | 1 | 13 | 11 | .. | .. | .. | 4 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Elliptio dilatatus | 7 | 63 | 7 | .. | 2 | 43 | 53 | .. | 2 | .. | 6 | 3 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Strophitus rugosus | 6 | 23 | 1 | 3 | 2 | 9 | 20 | .. | 4 | 14 | 1 | 7 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Anodonta grandis | 1 | .. | .. | 4 | 2 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Anodonta imbecillis | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Anodontoides ferussacianus | 2 | 2 | 4 | 3 | 3 | 1 | .. | 2 | .. | .. | .. | 5 | 15 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Lasmigona compressa | 1 | 3 | 1 | .. | 1 | .. | .. | .. | 5 | .. | .. | 1 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Lasmigona complanata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Lasmigona costata | 1 | 1 | .. | .. | .. | .. | .. | .. | 1 | .. | .. | 3 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Alasmidonta calceolus | 8 | 21 | 38 | 2 | 29 | 6 | 15 | .. | .. | .. | 1 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Alasmidonta marginata | 1 | 1 | .. | .. | .. | .. | 9 | .. | .. | .. | .. | 2 | 7 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Proptera alata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Leptodea fragilis | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Actinonaias carinata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Actinonaias ellipsiformis | 42 | 90 | 124 | 38 | .. | 25 | 37 | 66 | 1 | 10 | 5 | 13 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Micromya iris | 1 | 23 | 151 | 150 | 1 | 16 | 14 | 22 | 23 | .. | 2 | 11 | 1 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Ligumia recta latissima | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Lampsilis siliquoidea | 40 | 1 | 4 | 33 | 26 | 12 | 1 | .. | .. | .. | .. | 1 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Lampsilis ventricosa | 15 | 6 | 9 | .. | .. | 1 | 4 | 2 | 1 | 2 | 2 | 3 | 5 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Truncilla donaciformis | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Total no. of species | 13 | 13 | 13 | 9 | 8 | 12 | 10 | 8 | 4 | 7 | 9 | 9 | 8 | | | | | | | | | | | | |

| SPECIES | TRIBUTARIES | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50. Flat River, at Gowan | | | 51. Creek, outlet Lincoln Lake | | | 48. Flat R., s. of Belding | | | 47. 2 mi. ne. of Lowell | | | | | | | | | | | | | | | |
| Quadrula pustulosa | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Cyclonaias tuberculata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Amblema costata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Amblema peruviana | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Fusconaia flava | .. | .. | .. | 9 | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Pleurobema cord. coccineum | .. | .. | .. | 4 | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Elliptio dilatatus | 2 | .. | 6 | 3 | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Strophitus rugosus | 4 | 14 | 1 | 7 | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Anodonta grandis | .. | .. | .. | 1 | 1 | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Anodonta imbecillis | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Anodontoides ferussacianus | 2 | .. | .. | .. | .. | .. | 5 | 15 | .. | .. | .. | .. | | | | | | | | | | | | | |
| Lasmigona compressa | .. | .. | .. | 5 | .. | .. | .. | .. | 1 | .. | .. | .. | | | | | | | | | | | | | |
| Lasmigona complanata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Lasmigona costata | .. | .. | .. | 1 | .. | .. | .. | .. | 3 | .. | .. | .. | | | | | | | | | | | | | |
| Alasmidonta calceolus | .. | .. | .. | 1 | 1 | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Alasmidonta marginata | .. | .. | .. | .. | .. | .. | 2 | 7 | .. | .. | .. | .. | | | | | | | | | | | | | |
| Proptera alata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Leptodea fragilis | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Actinonaias carinata | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Actinonaias ellipsiformis | 1 | 10 | 5 | 13 | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Micromya iris | .. | 2 | 11 | 1 | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Ligumia recta latissima | .. | .. | .. | .. | .. | .. | .. | .. | 6 | .. | .. | .. | | | | | | | | | | | | | |
| Lampsilis siliquoidea | .. | .. | .. | 1 | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Lampsilis ventricosa | 1 | 2 | 2 | 3 | 5 | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Truncilla donaciformis | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | | | | | | | | | | | | | |
| Total no. of species | 4 | 7 | 9 | 9 | 8 | | | | | | | | | | | | | | | | | | | | |

III

IN THE GRAND RIVER

| | | |

Fig. 1. Distribution of *Actinonaias ellipsiformis* (circles) and *Lampsilis fasciola* (triangles) in southern Michigan

them, a remarkable set of correlations is evident. The rôle of glacial history in the distribution of *Lampsilis fasciola* has already been discussed (van der Schalie, 1938a, pp. 10–14). Consequently, only the conditions affecting the distribution of *Actinonaias ellipsiformis* will be considered at this time. It not only occurs in all the rivers on the west side of the state south of and including the Grand, but has also entered the tributaries of the Saginaw drainage basin (Fig. 1). This pattern of distribution is to be expected in view of present knowledge concerning the confluence that formerly existed between the Saginaw and Grand River drainages. According to Russel and

Leverett (1908, p. 13), that connection persisted while Lake Warren discharged its waters across the Saginaw–Grand divide. This confluence accounts for the invasion of the Saginaw River drainage by *ellipsiformis* and other river-inhabiting species of mussels. No explanation has yet been advanced, however, for the absence of *ellipsiformis* in the Muskegon River drainage. Both *Actinonaias carinata* and *Alasmidonta marginata* probably entered the Muskegon during the period when the Grand and Muskegon rivers were connected, as has already been discussed. The fact that *ellipsiformis* did not enter the Muskegon simultaneously may mean that it invaded the Grand River drainage at a period when the connection between that river and the Muskegon no longer existed, but before the severance of the confluence between the Saginaw and Grand River drainages.

There is strong evidence that faunal differences between areas should be explained on the basis of glacial history rather than by ecological differences in the habitats provided by the areas. A recent textbook entitled *Ecological Animal Geography* (Hesse, 1937, p. 132) contains the statement that "broader areas, with other conditions equal, in general have a richer fauna. A principal reason may be that they afford a wider range of habitat conditions, and thereby increase the possibilities for the formation of species by adaptation." It is obvious, however, that such reasoning would hardly explain the large difference between the number of naiades at present found in Lake Erie, which has thirty species, and in Lake Michigan, which has, perhaps, a maximum of ten. Nor would ecology by itself be a sufficient reason for the faunal differences observed in the Grand and Muskegon rivers (Table I).

Nevertheless, a knowledge of the ecology of the various species of naiades has proved of considerable value in an interpretation of the origin of present distribution patterns of species within the different drainages. Figure 2 and Tables II–III are submitted to show graphically and in condensed form the ecological and distributional data on naiades in the Grand and Muskegon rivers. The information contained in the tables will be summarized here.

As has already been suggested, the naiades of the Muskegon River constitute an aggregation which is clearly that of a former tributary of the Grand River. This relationship becomes evident if one compares the faunal list in Table II (Muskegon River species) with that in Table III for the Red Cedar drainage, which is at present

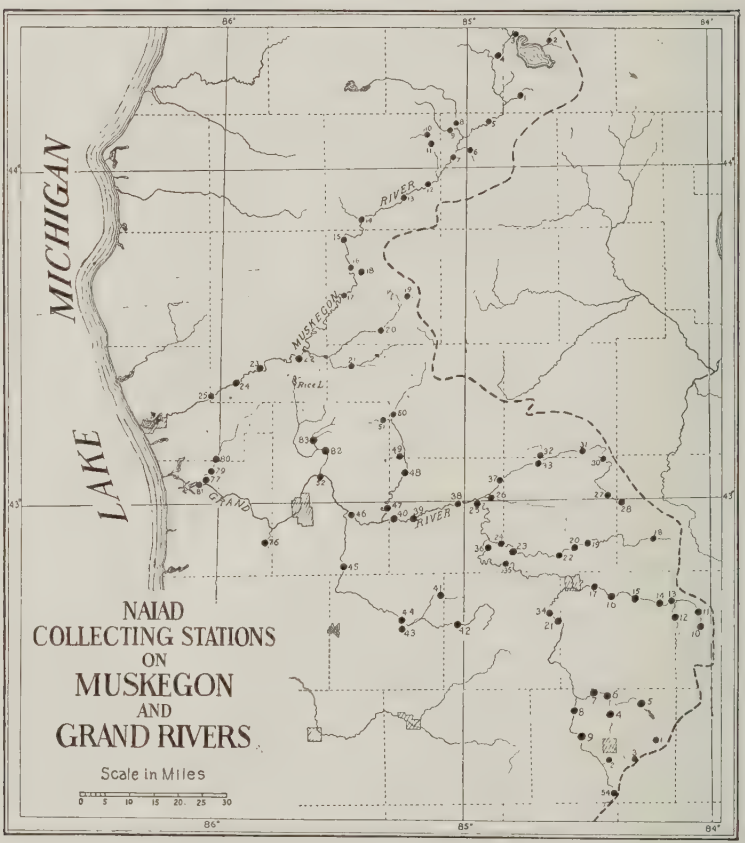


Fig. 2

a tributary of the Grand River. These lists are so nearly identical that the faunas of the Muskegon River and the Red Cedar River show virtually the same relation to the Grand River fauna. It will be noted, however, that four species occurring in the Muskegon River are absent from the Red Cedar. The presence of two of them, *Proptera alata* and *Dysnomia triquetra*, in the Muskegon River is obviously accounted for by the fact that they invaded the lower Muskegon River from Lake Michigan. The other two species, *Actinonaias*

*carinata* and *Ligumia recta latissima,* are ecologically large-river forms (see Table III), which are in the Muskegon because at one time it joined the lower portion of the Grand. The ecological evidence tends to confirm the view that the Rouge River valley served as the former line of junction between these major drainage systems. Further evidence that species usually associated with lower reaches of a river may invade smaller tributaries near the mouth of the river is seen in the occurrence of *Quadrula pustulosa,* a large-river species (Table III), which was found near the mouth of Maple River and Crockery Creek. In addition, the invasion of Crockery Creek by *Lasmigona complanata, Proptera alata, Leptodea fragilis,* and *Actinonaias carinata* also illustrates this point. As is shown in the tables, none of these species are associated with creeks farther upstream.

Since the naiad fauna of the Muskegon River so closely approximates the fauna inhabiting the larger tributaries of the headwaters in the Grand River, it is possible to group the naiades of both drainages into the following ecological assemblages:

Three species common to creeks:

Lasmigona compressa — Anodontoides ferussacianus
Alasmidonta calceolus

Fourteen species occurring under medium-sized–river conditions:

Fusconaia flava — Alasmidonta marginata
Elliptio dilatatus — Lasmigona costata
Pleurobema cordatum coccineum — Micromya iris
Amblema costata — Ligumia recta latissima
Strophitus rugosus — Lampsilis siliquoidea
Anodonta grandis — Lampsilis ventricosa
Anodonta imbecillis — Actinonaias ellipsiformis

Five species common under the large-river conditions found in the lower portion of the main stream:

Quadrula pustulosa — Amblema peruviana
Cyclonaias tuberculata — Actinonaias carinata
Ligumia recta latissima

Several other naiades are clearly associated with the extreme lower reaches of both drainages because of conditions obtaining there. These studies, as well as those of other drainages in Michigan, have shown that the following species are invaders from Lake Michigan:

Lasmigona complanata — Leptodea fragilis
Proptera alata — Truncilla donaciformis
Dysnomia triquetra

Four other species found only below Grand Rapids, reported by Coker and others (1921, p. 95), should be added to the last group:

| | |
|---|---|
| Quadrula quadrula | Obovaria olivaria |
| Obliquaria reflexa | Truncilla truncata |

With the exception of the differences just listed the naiades of the Grand River reported by Coker and others (1921) are essentially the same as those shown in Table III. Dredging operations in the lower Grand River between Grand Rapids and Grand Haven have been largely responsible for the present scarcity of the mussels in that zone.

Since many factors are causing the depletion of the indigenous fauna, it becomes increasingly important that information regarding ecology and distribution be gathered before the damage becomes too extensive. In the Grand River other agents besides dredging are and have been particularly harmful. Sewage and many kinds of industrial wastes have entered it below Jackson, Lansing, and Grand Rapids. There has been in addition an excessive amount of mussel-shell gathering with the use of apparatus injurious to the mussel beds (van der Schalie, 1938b). The power dams in the Muskegon River are most harmful to the naiades of that drainage, since species normally inhabiting a stream are usually eliminated from the impounded water behind dams. Such obstructions also hinder the normal migration of fishes, the carrying agents for fresh-water mussels. Lumbering activities in the headwaters and pollution near the mouth have likewise contributed toward eradicating naiades in the Muskegon River.

Variation in the nacreous color of certain mussels has always been of interest. Most species have a white nacre, but in several the nacre is consistently purple or pink. Occasionally a species, such as *Elliptio dilatatus*, which is normally purple, will produce white-nacred specimens. In this general connection it was particularly striking that so many white-nacred specimens of *dilatatus* were found in the Muskegon River. There is no conclusive information about whether nacreous color is a response to environmental conditions or is due to genetic factors. Table IV shows the relative proportion of white to colored specimens of *dilatatus* in the Muskegon River.

The proportion of specimens with colored nacre to those with

TABLE IV

NACREOUS COLOR VARIATION OF *ELLIPTIO DILATATUS* AT STATIONS IN THE MUSKEGON RIVER DRAINAGE

| Station number | Location of station | Nacre | | Total |
|---|---|---|---|---|
| | | Purple | White | |
| 2 | River, between Houghton and Higgins lakes | 16 | 17 | 33 |
| 3 | River, 3 miles below outlet of Houghton Lake | 43 | 28 | 71 |
| 12 | River, 11 miles south of Marion | 14 | 6 | 20 |
| 13 | River, 1 mile northeast of Evart | 56 | 19 | 75 |
| 14 | River, at junction with Hersey Creek | 36 | 13 | 49 |
| 15 | River, above Paris | 29 | 26 | 55 |
| 18 | Ryan Creek, 2 miles southeast of Big Rapids | 19 | 11 | 30 |
| 20 | Little Muskegon River, near Altona | 8 | 5 | 13 |
| Total | | 221 | 125 | 346 |

white nacre is almost two to one. All available evidence (van der Schalie, 1938a, pp. 48–49) indicates that there is practically no correlation of nacreous color with the range of the species. Further studies on the genetics of the color may prove more successful in solving the problem of causal relations responsible for this variation.

SUMMARY

The zoogeography of the Mississippian naiad fauna of southern Michigan is best explained in terms of glacial history. Naiades reached southeastern Michigan from the Mississippi River by way of the Wabash and Maumee rivers; the species in streams of southwestern Michigan came by way of the Des Plaines River and the Chicago outlet to the Mississippi. Faunal evidence indicates that the Muskegon River was formerly a tributary of the Grand. Ecological and distributional data support the contention that the Rouge River valley formerly connected the Muskegon River with the Grand. Twenty-nine species of fresh-water mussels inhabit the Grand River; twenty-one are reported in the Muskegon. The species are grouped ecologically into four major assemblages.

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# THE HERMAPHRODITE GLAND AND GERM CELLS OF *VALLONIA PULCHELLA* MÜLL.*

MARGARET ESTHER WHITNEY

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* Contribution from the Department of Zoölogy, University of Michigan.

## INTRODUCTION

THE numerous studies of hermaphrodite mollusks have brought to light a variety of interesting and unusual reproductive conditions. Of particular interest relative to this paper is the capacity for self-fertilization revealed by some of the forms studied. Though in general hermaphroditism in this group has been regarded as of the "insufficient" type, the laying of fertile eggs in strict isolation has been recorded in a number of instances. Ikeda (1937) lists reports of this kind covering thirty species of Pulmonata, belonging to ten different genera. Some authors have questioned how far these laboratory results could be considered indicative of natural processes. For the most part they have been obtained with animals which copulate freely in nature.

The study presented here concerns *Vallonia pulchella* Müll., a small land snail, which gives evidence of a natural physiological isolation in contrast to one artificially brought about. Steenburg (1918) and Watson (1919) describe the hermaphrodite organs from minute dissections and call particular attention to the absence of male ducts in the majority of the specimens examined. A similar reduction of male organs has been found in *Acanthinula aculeata* (Boycott, 1917; Steenburg, 1918) and also in *Bullinus contortus* (Larambergue, 1930, 1932, 1933). In some cases the aphallic state is interpreted by Boycott as being a structural simplification associated with diminution in absolute size.

Elimination not only of male ducts but of male cells, resulting in parthenogenesis, has been reported for *Paludestrina jenkinsi* by Robson (1923) and for *Campeloma rufum* by Van Cleave and Altringer (1937) and Mattox (1937).

In a preliminary paper I have described the egg-laying habits of *Vallonia pulchella*, the hatching of the eggs, and the growth of the young to maturity (Whitney, 1938). Under laboratory conditions isolated individuals laid an average of one egg a day over a period of several months. The young were well developed and active when hatched. Desiccation and low temperatures were found to have an important modifying effect upon the egg-laying function and upon development. Copulation was never observed. Individuals isolated from the time of hatching laid eggs from which young hatched, and some of the latter reared in isolation reproduced

in like manner. This observation continued for several successive generations.

These macroscopic observations formed a basis for the present microscopic study to determine further details of reproduction in *Vallonia pulchella*, particularly those relating to self-fertilization. The possibility of parthenogenesis was not entirely excluded, even though male cells were present, since in many hermaphrodites various barriers against self-fertilization exist.

Some of the interesting phases of the problem concerned the origin and differentiation of male, female, and nutritive cells; possible abnormalities of maturation resulting in atypical sperm and ova, such as have been reported for other mollusks; possible cyclic conditions; modifications imposed by the small size of the animal, and by environmental factors; and, finally, the survival value of the conditions found, so far as this could be inferred. Self-fertilization and parthenogenesis have been cited as contributing factors in bringing about wider distribution of certain molluscan forms, especially under the influence of civilization (Robson, 1923; Boettger, 1931). Observations of *Vallonia* suggested similar interpretations.

I take this opportunity to express appreciation to Professor Peter Okkelberg for having directed this study, to members of the Departments of Zoölogy and Botany and of the Museum of the University of Michigan for valued suggestions and assistance, and to members of the Botany and Zoölogy Departments of Butler University, Indianapolis, Indiana, for many favors.

## GENERAL REVIEW OF THE LITERATURE

The problems studied necessitate a review of some of the literature bearing on hermaphroditism and self-fertilization among the mollusks in general and the pulmonates in particular. References having to do with more detailed aspects of the study will be reserved for sections in which they apply.

Furrow (1937), reviewing the work on sex in the Mollusca, distinguishes two types of hermaphrodite glands: the "segregated," in which there is an anatomical separation of male and female elements; and the "unsegregated," in which male and female cells arise within the same cysts. He points out, however, that in the latter there is a segregation in the time of development of male

and female cells which prevents self-fertilization. The intervals vary considerably, and under experimental conditions may be shortened almost to the point of complete elimination, when self-fertilization becomes possible. Furrow cites examples of such experimental alterations of the sexual cycle in the pond snails *Planorbis* and *Lymnaea*. Crabb (1927, 1928), however, expresses the view that self-fertilization is the normal, not the unusual, method of reproduction in the species of *Lymnaea* which he studied.

There has been some disagreement regarding barriers against self-fertilization. If such barriers are postulated, production of fertile eggs by isolated hermaphrodites must be attributed to parthenogenesis. As evidence in favor of self-fertilization rather than parthenogenesis in some of the forms studied authors have cited the presence of ripe sperm and eggs in the same cysts at practically all times, the giving off of two polar bodies, the development of two pronuclei, and the numerical ratios exhibited by the offspring of isolated heterozygotes (Crabb, 1927; Larambergue, 1930; Ikeda, 1937). In the parthenogenetic snail *Campeloma rufum*, in which no male elements whatever were to be found, Mattox (1937) describes a type of oögenesis in which only one polar body is produced, with no evidence of synapsis or reduction.

In general, one gathers from a survey of the literature that conditions in many hermaphrodite mollusks are such as to permit self-fertilization in varying degrees, and that in some this phenomenon may possibly occur naturally in combination with cross-fertilization.

## MATERIALS AND METHODS

For this study specimens of *Vallonia pulchella* Müll. were collected from typical locations in Ann Arbor, Michigan, and Indianapolis, Indiana. Some were reared in the laboratory. Microscopic preparations were made of adult specimens taken throughout the year, with a view to correlating histological conditions of the ovotestis with the seasonal activity observed. In order to trace the development of the gland juvenile specimens of different sizes were prepared. This series was graded from 0.6 mm., the size of individuals measured shortly after hatching, to 2.0 mm., the average adult size — the measurements representing the widest shell diameter of alcohol specimens.

The material was fixed in modified Bouin's solution, which de-

calcified the shell, so that it was easily removed. Normal butyl alcohol was used in the dehydration and infiltration of some of the specimens and dioxane for the rest. Serial sagittal sections gave the most favorable disposition of the organs for study. The material was sectioned for the most part at 8 or 10 microns, and stained with Heidenhain's iron hematoxylin without counterstain.

The small size was of some advantage for microscopic study in that a complete individual series could be accommodated on one or two slides, and the entire hermaphrodite gland could be studied *in situ*. In larger forms it must be removed and divided.

The figures were prepared from photomicrographs and from camera lucida drawings.

## ANATOMY OF THE HERMAPHRODITE ORGANS

The hermaphrodite organs of *Vallonia*, as described by Steenburg (1918), include the hermaphrodite gland, the hermaphrodite canal, the seminal canal, which is continuous with the vas deferens and penis, the narrow oviduct and vagina, and the genital cloaca, which opens by way of the common genital orifice behind the right tentacle. The prostate and the albumen gland and other accessory structures are also described. We have mentioned above that both Steenburg (1918) and Watson (1919) found the penis and vas deferens lacking in many specimens. This observation was confirmed by the present study, since careful examination of sections failed to reveal anything resembling these structures.

The hermaphrodite gland, or ovotestis, is embedded in the lower portion of the upper division of the liver. Steenburg (1918) describes its form as follows: "The pyriform acini are united in three groups, not very distinct, comprising three or four acini each." A gonad of this description is of the "unsegregated" type as distinguished by Furrow (1937). The cysts containing both male and female germ cells join the hermaphrodite duct, which serves as both a sperm duct and an oviduct. This condition is found in *Helix*, *Physa*, *Lymnaea*, *Planorbis*, and *Polygyra*, as well as in *Vallonia*.

## HISTOLOGY OF THE ADULT GLAND

Sagittal sections of mature animals collected during the active season show quite clearly the three groups of acini making up the ovotestis, as described by Steenburg (Pl. I, Fig. 1). The cortical

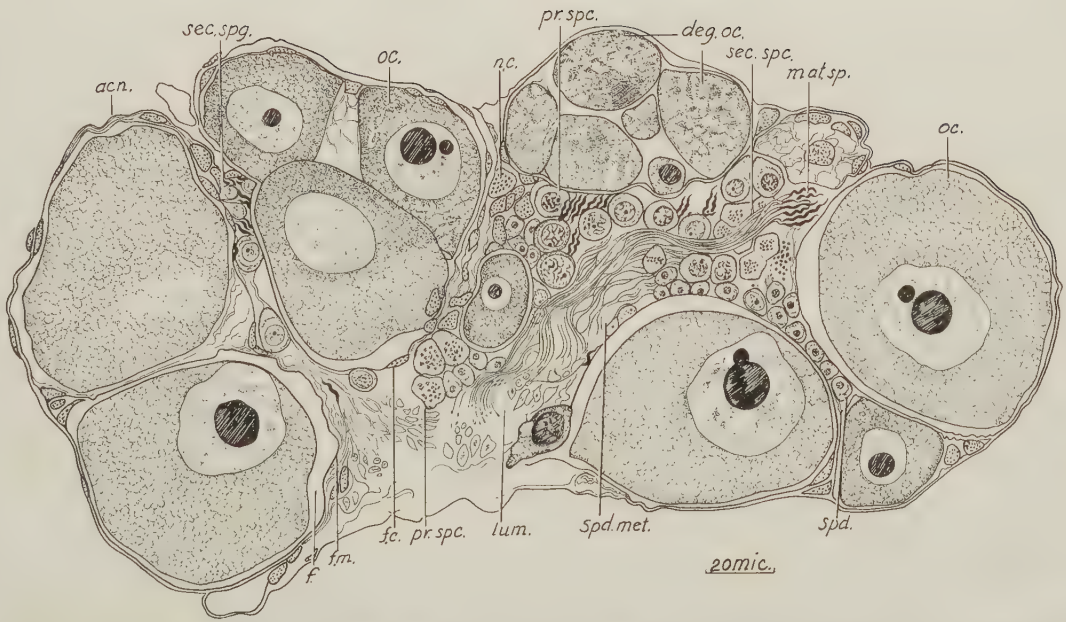


FIG. 1. Camera lucida study of mature gonad in individual collected in July. Abbreviations: *acn.*, acinus wall; *deg. oc.*, degenerating oöcytes; *f.*, follicle; *f. c.*, follicle cell; *f. m.*, follicular membrane; *lum.*, lumen; *mat. sp.*, mature spermatozoa; *n. c.*, nutritive cell; *oc.*, oöcytes; *pr. spc.*, primary spermatocytes; *sec. spc.*, secondary spermatocytes; *sec. spg.*, secondary spermatogonia; *spd.*, spermatids; *spd. met.*, spermatids in metamorphosis. (Reconstructed from three adjacent sections)

regions are crowded with large oöcytes protruding from the walls of the acini (text Fig. 1). These are covered with thin follicular membranes, in which at intervals isolated nuclei of various sizes appear. Some of the smaller nuclei are much flattened. The larger ones, of the nurse-cell type, are more rounded, and their chromatin has the characteristic appearance of coarse angular blocks. Cell outlines are difficult to distinguish. According to Gatenby (1919a) and Woldt (1932), the large "hyperchromatic" nurse cells apparently develop from smaller epithelial cells of more generalized type. Gatenby found all gradations between them.

It has been suggested that these nutritive or follicle cells, by building the follicular membranes, prevent self-fertilization; and again that they serve as supporting cells, holding the ova in position so that they remain advantageously situated with respect to the nutritive fluids of the body cavity, which must diffuse from the outside to the interior of the gland.

In *Vallonia*, as in *Helix* and other forms, it seems probable that additional nutritive material is contributed by degenerating oöcytes, which are very commonly found in the ovotestis. Some sections may show the whole contents of a cyst made up of these (text Fig. 1), or a cyst may contain one large developing oöcyte and several smaller degenerating ones.

Additional nutritive material may be contributed by spindle-shaped fragments of cytoplasm often observed in the neighborhood of mature spermatozoa and spermatids in various stages of metamorphosis (text Fig. 4p). It seems likely that this material, discarded during the process of metamorphosis, helps to form a nutritive fluid which fills the interior of the gland and bathes the cells.

The lumen of the active gland is occupied by male cells in various stages of maturation, especially in the medullary portion, where the acini converge toward the hermaphrodite duct. This seems to be the most active region in the production of new elements, for here small new oöcytes also appear in the germ wall, in proximity to the male cells in the lumen. From this position the small oöcytes apparently migrate, as they grow, into the deeper portions of the acini. Many of them exhibit an elongated, tapering form which suggests an ameboid migration, somewhat similar to that described for the germ cells of *Planorbis* by Merton (1930).

The male cells tend to occur in groups, although some isolated ones are scattered about, here and there. The cells composing each group are all in approximately the same stage of development, a condition noted in *Helix* by Demoll (1912) and others. Such groups have been called "cell nests," and each one has arisen, according to interpretation, from a single primary spermatogonium.

Fifteen to twenty large oöcytes, besides a number of smaller ones, have been counted in typical specimens collected in July. These quite fill up the acini and, together with the groups of male cells crowding the lumen, give the whole gland a relatively compact appearance.

Sections of material fixed during the dormant season, in January, for example, show the gland in a somewhat ragged form, with open spaces left by the discharge of cells during the previous season (Pl. II, Fig. 2). A number of large oöcytes may be left over, and some of medium size, but few if any small new ones are to be found. Male cells are few and scattered, not exhibiting the orderly arrangement in groups which is indicative of recent division. Throughout the gonad many of the cells appear shrunken and distorted, but others do not differ markedly from corresponding stages found during the active period. Conceivably they are in an arrested condition and might regain activity and function the following season.

Although egg laying did not take place in the out-of-door sites to any great extent until the middle of May, individuals collected early in March and April show evidence of considerable growth activity in the hermaphrodite gland, doubtless in response to warmer temperatures during a part of the day. In particular, the active medullary region is occupied by a considerable number of small oöcytes at the onset of their growth, and by early spermatocytes as well (Pl. II, Fig. 3).

Material fixed in the fall shows fewer cells and more open space (Pl. II, Fig. 4) than the summer material. Later growth and maturation stages are more conspicuous than earlier ones.

Animals reared or kept for some time in the laboratory under what might be regarded as optimum conditions are characterized in section by the same abundance and variety of growth and maturation stages as those collected during the active season out of doors.

THE DEVELOPING GLAND

The classic work of Ancel (1902) on *Helix pomatia* describes the genital rudiment as it appears within the mesoderm several days before hatching. At this early stage it is a quite solid mass of cells, which slowly elongates and ultimately fuses with the hermaphrodite canal. A lumen then appears which transforms the gonad into a hollow vesicle, united at one end with the hermaphrodite duct and expanded at the other. In Ancel's opinion, all the cells making up the genital gland are derived from elements of the primordial anlage. At no time do foreign elements penetrate into it. According to his observation, sperm cells were the first to differentiate, then nurse cells, and then egg cells, but Buresch (1911) describes these three elements as arising simultaneously from the germinal epithelium.

Pennypacker (1930) found both male and female germ cells in the ovotestis of *Polygyra appressa* from the 5-mm. stage on. These authors note that mature spermatozoa are present in the young gland of both *Helix* and *Polygyra* long before mature ova, and that the development of the male cells takes place in the lumen, whereas the female cells remain in the walls of the acini during their entire stay in the ovotestis.

The early development of the hermaphrodite gland of *Vallonia pulchella* presents striking similarities to that described by Ancel for *Helix*. In the present study the genital rudiment was first identified about the time of hatching. The stages representing the course of development are described and figured (Pl. III, Figs. 1–7). The time required to complete development varies somewhat with external conditions, so that size was regarded as a better descriptive criterion of the series than age.

*The 0.6-mm. Stage* (Pl. III, Fig. 1; Pl. IV, Fig. 1; text Fig. 2)

The newly hatched animal measures 0.5 to 0.6 mm. in diameter. It emerges well developed and active, with a whorl of its body shell completed. At this time the hermaphrodite gland is a small compact mass of dense, closely clustered cells, situated in the inner coil of the body. The cells are very small, scarcely more than 5 microns in diameter, and have proportionately large nuclei and very little cytoplasm. They are essentially alike in appearance, although varying somewhat in size. It is not possible at this stage to recognize

FIG. 2. Camera lucida study of immature undifferentiated gonad at time of hatching. Abbreviations: *h. d.*, hermaphrodite duct; *prim. g. c.*, primordial germ cells; *sh.*, sheath of gonad. (Reconstructed from two adjacent sections)

which cells will mature into male and which into female germ cells. In some preparations they appear grouped in a few small grapelike clusters, as if they had arisen by division from a still smaller number of cells of earlier origin. The whole group is invested with a sheath containing small darkly staining nuclei. Ventrally the genital rudiment tapers off into the very slender, slightly wavy hermaphrodite duct, which at this time also appears to be a solid rudiment. This stage reminds one of the first small mass of cells described by Ancel (1902) for *Helix*. Hsiao (1939) describes a similar undifferentiated early gonad for the marine snail *Limacina retroversa*.

*The 0.9-mm. Stage* (Pl. III, Fig. 2; Pl. IV, Fig. 2)

In animals measuring 0.9 mm. the massive arrangement of the gland persists, but cell differentiation is now initiated. At the periphery of the mass a few cells show the beginning of growth and are clearly recognizable as oöcytes because of the proportion of

cytoplasm to nucleus and the prominent double nucleoli, which are characteristic of oöcytes both large and small wherever found in the gland. At the center of the group are smaller cells, many with interphase nuclei, but a number in prophase and some with distinct chromosomes. These are the primary spermatogonia, and sometimes there are associated with them still smaller cells of similar appearance, the secondary spermatogonia. Thus the characteristic orientation of female cells toward the periphery and male cells in the interior becomes evident in the very young gland, in which no definite lumen is as yet formed. A few nurse-cell nuclei are irregularly disposed toward the outer portion of the cell group.

*The 1.0-mm. Stage* (Pl. III, Fig. 3; Pl. IV, Fig. 3; text Fig. 3)

In most preparations of 1.0-mm. animals or those a little larger there is a definite lumen in the hermaphrodite gland, so that it has the form of a simple undivided vesicle, which tapers below into the hermaphrodite duct. One is impressed with its similarity to the early gonad of *Helix* as described by Ancel. The older oöcytes are larger than those of previous stages, and small new ones occur in the portion of the gland nearest the hermaphrodite duct. As they grow they apparently migrate toward the deeper expanded portion of the germinal vesicle, in which region the largest ones are found. In the lumen are male cells in various stages. A number of ripe spermatozoa are included. Their early appearance in *Helix* and *Polygyra* has been noted.

There is evidence of an occasional penetration of these early maturing sperm cells into young oöcytes. At least some sections show bodies in the egg cytoplasm which are in every way similar to surrounding sperm heads, and at the periphery of these oöcytes are sperm heads which give the appearance of having just penetrated the surface by means of spiral movements (Pl. IV, Fig. 3; text Fig. 3). It is difficult to say what such an early impregnation may signify. In the slug *Arion empiricorum*, Lams (1910) has noted that oöcytes are regularly inseminated while still in the germ wall, and maturation and cleavage stages occur in the interior of the gland. Buchner (1914) describes a penetration of spermatozoa into the early oöcytes of the archiannelid *Saccocirrus*, the sperm being retained in the egg cytoplasm throughout the growth period and then taking part in an entirely normal fertilization.

FIG. 3. Camera lucida study of immature gonad from 1.0-mm. individual, showing presence of mature spermatozoa and their penetration into young oöcytes. Abbreviations: *h. d.*, hermaphrodite duct; *lum.*, lumen of the gland; *mat. sp.*, mature spermatozoa; *n. c.*, nutritive cell; *oc.*, oöcyte; *pr. spg.*, primary spermatogonia, interphase and metaphase stages; *sec. spg.*, secondary spermatogonia; *sh.*, sheath. (Reconstructed from two adjacent sections)

In *Vallonia* it has not been possible to trace any definite sequence of events following early impregnation. It seems improbable that it can have any significance in fertilization; rather, the fate of these early penetrating spermatozoa is a rapid absorption. At times indefinite elongated bodies are seen in oöcytes of various sizes which have somewhat the appearance of sperm cells in process of dis-

integration, although other interpretations are possible. They are seldom found in full-grown oöcytes, and it seems likely that any penetration in early stages may be due to the follicular membranes not being well formed, and that when these are fully developed they make an effective barrier against insemination of the egg until after ovulation. I have never discovered any cleavage stages or even maturation spindles in oöcytes while they remained in the ovotestis. It seems clear that the definitive insemination resulting in fertilization does not occur until the female cells have become detached from the acinus walls.

*The 1.3-mm. Stage* (Pl. III, Fig. 4)

The principal changes noted in the 1.3-mm. stage are a widening and upward extension of the gland and the development of a more lobate form. There is also an increase in size of the oldest oöcytes as compared with those of earlier stages, and with the widening of the lumen the characteristic grouping of male cells in "cell nests" becomes apparent.

*The 1.5-mm. Stage* (Pl. III, Fig. 5)

In the 1.5-mm. stage a division into primary acini is indicated, accompanied by a further increase in size of the oöcytes and of the gland as a whole.

*The 1.8-mm. Stage* (Pl. III, Fig. 6)

In animals measuring 1.8 mm. there occur further enlargement and a subdivision of the gland into lobules, and the oöcytes approach the maximum size of those in the adult gland. New generations of smaller oöcytes continue to appear in the active medullary region. The lumen is almost completely filled with groups of developing male cells representing practically all stages of maturation.

*The 2.0-mm. Stage* (Pl. III, Fig. 7; Pl. IV, Fig. 4; text Fig. 1)

Two millimeters is the average adult size, and when the young animal reaches this diameter the ovotestis corresponds in structure to that already described for the active mature individual. Egg laying follows very shortly, as was observed in my previous study (Whitney, 1938), so that sections may show not only large oöcytes filling up the acini of the hermaphrodite gland but a fertilized egg in the outer genital passage, invested with albumen and shell and ready to be laid (Pl. I, Fig. 2).

MATURATION OF THE GERM CELLS

The maturation of the germ cells of Pulmonata and of other hermaphrodite mollusks has been the subject of a great amount of careful investigation. Since the present study does not deal with detailed observations of chromosome structure, or of the nebenkern, mitochondria, or other cytoplasmic inclusions, no attempt will be made to review this extensive literature in any detail. Most authors agree on the origin of male, female, and nutritive cells from an indifferent germinal epithelium. Both internal genic factors and external influences, such as proximity to nutritive sources, have been cited as sex-determining (Ancel, 1902; Buresch, 1911; Gatenby, 1917, 1918, 1919a and b; Pennypacker, 1930).

Demoll (1912), Alexenko (1928), and Perrot (1930) have described heterochromosomes and have followed their course through spermatogenesis and oögenesis. The manner in which these operate to determine sex remains, however, somewhat hypothetical. A considerable section of the literature has to do with the production of atypical spermatozoa and abortive ova. This literature has been reviewed by Furrow (1935, 1937), who summarizes the chief theories concerning the significance of these abnormal forms. In some studies atypical spermatozoa have been found to effect a partial fertilization (Hyman, 1925; Portmann, 1930). By penetration or contact they seem to determine which eggs are destined to have a merely nutritive function. The usual fate of abnormal spermatozoa, however, is degeneration, and, on the whole, Furrow is inclined to regard them as the result of the interaction of two widely differing germinal tissues in the same gland. They represent a sort of rudimentary hermaphroditism which might be expected in forms intermediate between hermaphroditism and definite dioecism. This is found even in higher animals such as the frog, which in some instances shows a tendency to develop ovarian tissues in the testes. According to Ankel (1930), this labile sex condition would be expected to result in dimorphism of eggs as well as sperm, a dimorphism manifested by an inability of a certain number of eggs to develop. He points out that degenerating eggs are very common in prosobranch ovaries and sometimes serve as nutritive material.

In *Vallonia*, though there is some variety in the appearance of the cells and though unusual-looking spermatozoa have now and

then been found, no regular dimorphic series have been identified. If such dimorphism exists, the atypical forms must be quite rare. The majority of cells observed can be readily classified as belonging to the normal spermatogenetic series. Degenerating ova are common, as previously mentioned.

### *Spermatogenesis*

The spermatogenesis of *Vallonia pulchella* is apparently typical. The stages are figured (text Figs. 4a–r), so that only their general features will be discussed.

*Spermatogonia.* — The first male cells identified in the interior of the young gland, while it is still in the form of a solid rudiment, are interpreted as being primary spermatogonia (text Figs. 4a–c). They are less easily found and identified in later stages, since they tend to occur singly and are not numerically prominent among the maturation stages. As compared with the indifferent cells of the earlier period, they are large, having a long diameter of 8–10 microns. They are frequently pedicellate, with the base directed toward the germinal epithelium or follicular membrane, and the wider portion of the cell, which contains the nucleus, facing the lumen. A number of them show mitotic figures, but chromosomes are difficult to count, owing to their small size and crowded condition. The counts made ranged from 36 to 38.

Smaller spermatogonia, 5–6 microns in diameter, are identified as secondary, being derived from the primary stages by mitotic divisions (Figs. 4d–e). Their chromatin is less finely dispersed, and they have a more rounded and compact appearance. They occur in groups suggesting recent divisions, whereas the primary spermatogonia, as stated above, are more likely to be found singly.

Primary spermatogonia are interpreted as originating from germinal epithelial cells, by a process of growth and differentiation. They are the first male cells shed into the lumen, where they give rise to secondary spermatogonia by repeated mitoses.

*Spermatocytes.* — Primary spermatocytes are numerous in mature glands during the active season. They are large cells 10–12 microns in diameter, and, like the spermatogonia, may be pedicellate. In section the cytoplasm forms a narrow border around the large nucleus. Typical preleptotene, leptotene, and bouquet stages are found (Figs. 4f–h). The tetrad stages (Fig. 4i) offer the best oppor-

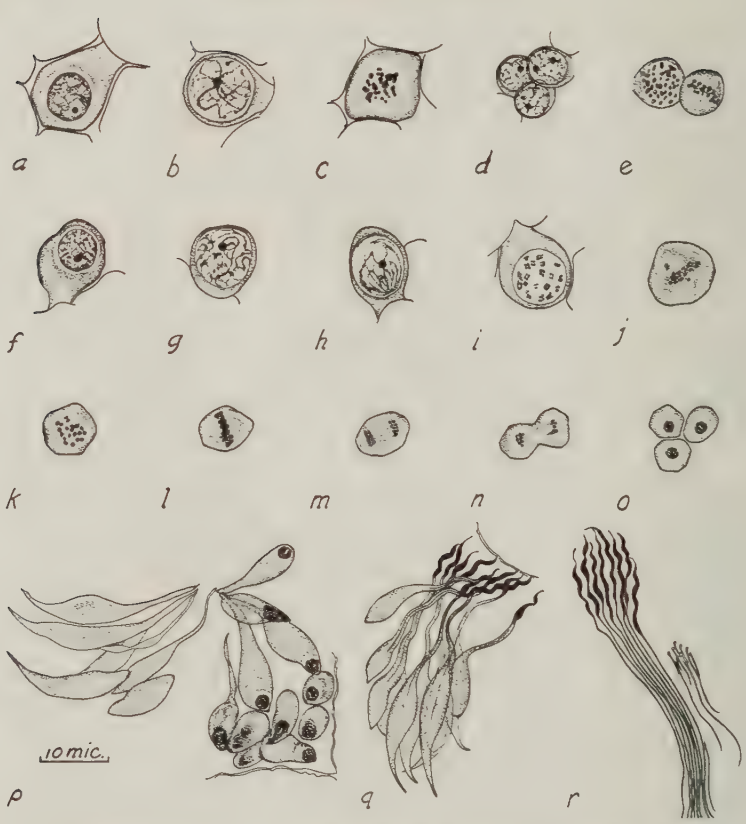


FIG. 4. Spermatogenesis: *a*, primary spermatogonium; *b*, primary spermatogonium in prophase; *c*, chromosome plate of primary spermatogonium, semiprofile view; *d*, group of secondary spermatogonia; *e*, chromosome plates of secondary spermatogonia; *f*, primary spermatocyte-preleptotene stage; *g*, leptotene stage; *h*, bouquet stage; *i*, tetrad stage; *j*, first maturation division, profile view; *k*, chromosome plate of secondary spermatocyte; *l*, second maturation division, profile view of metaphase; *m*, anaphase; *n*, telophase; *o*, spermatids; *p*, spermatids beginning metamorphosis; *q*, late stage of spermateliosis; *r*, group of mature sperm heads. (Selected from sections shown in Plate IV and text Figs. 1 and 3 and from other sections)

tunity for chromosome counts, since the chromosomes are better spaced in the large nucleus than in any other stage. On the average, about 20 tetrads have been counted.

Maturation divisions (Figs. 4j–n) evidently occur in rapid succession without the intervention of a resting stage, to judge from the infrequency with which these stages are encountered. These findings correspond with those of Demoll (1912) for *Helix*. Profiles of the two divisions are rather similar except for the differing sizes of the cells (Figs. 4j, l). The secondary spermatocytes measure 8–9 microns and show the small rounded chromosomes somewhat closely grouped in a dense homogeneous cytoplasm, without a nuclear membrane (Fig. 4k). A few chromosome counts were obtained. They ranged from 18 to 20.

*Spermatids.* — The spermatids (Figs. 4o–p) are small cells, 6–7 microns in diameter, and somewhat angular in outline as compared with cells of previous stages, probably owing to their being closely massed together in rather large groups often consisting of several dozen cells. They evidently remain in this stage for a considerable time, and are readily identified because of their uniform and characteristic appearance. They constitute a reserve from which a few mature spermatozoa are formed by metamorphosis from time to time.

*Spermateliosis* (Figs. 4p–r). — The cells are so small that little can be said of the details of metamorphosis. The spermatid elongates; the nucleus assumes a tapering form and comes to occupy one end. In some preparations an axial fiber is faintly visible. In later stages a spiral twisting of the sperm head occurs, a development described for other forms also (Byrnes, 1900; Woldt, 1932), and a spindle-shaped mass of cytoplasm is discarded from the tail. These discarded fragments apparently disintegrate, and may contribute to the nourishment of the maturing cells, as mentioned above.

*Spermatozoa* (Fig. 4r). — The mature spermatozoa, including the tail, may have a length of 60–70 microns. They usually occur in relatively small groups of about a dozen cells, but there are isolated ones also in various parts of the gland lumen. The tail is infrequently seen. It is probably lost before the sperm cell becomes functional, as authors have reported for other mollusks. We have already noted that spermatozoa may be present in the hermaphrodite glands of juvenile animals in which oöcytes have reached only a fraction of the maximum size.

### *Oögenesis*

*Origin of the female cells.* — Ancel (1902) claims a unicellular origin for the egg cell of *Helix*. He expresses the view that each

female cell is formed directly by a process of growth and differentiation from a germinal epithelial cell, without a preliminary multiplication through oögonial divisions. To quote his account, "As soon as the female cell is recognizable . . . it is in the growth stage, — one cannot differentiate in the young gland of *Helix* a female cell that is not an oöcyte. The oöcyte arises directly from an epithelial cell to which one cannot give at any time the name primordial ovule or ovogonium." Essentially the same opinion is recorded by Buresch (1911), Gatenby (1917), and Pennypacker (1930). Although Pennypacker found certain cells in *Polygyra* which she was inclined to interpret as being oögonia, she could not identify them with certainty. Buresch points out that in general the function of the oögonial divisions is to increase the number of eggs and thereby the chances of survival, but in *Helix* he notes other protective features which make the production of a large number unnecessary: the eggs are enclosed in a hard shell; they are deposited deep in the earth; and the young emerge well developed, protected by the body shell.

In some mollusks oögonial divisions have been reported, but in *Vallonia* the situation evidently agrees with that in *Helix* and *Polygyra*, and the female cells are first recognized as oöcytes. No definite cells can be distinguished as true oögonia. Few eggs are produced, and various protective features associated with egg laying and development may be postulated as making a large number unnecessary, much as in the case of *Helix*.

*Growth period.* — The first recognizable oöcytes are found in the periphery of the young gonad while it is still in the early compact stage (Pl. IV, Fig. 2). They stand out from the spermatogonia because of the larger proportionate size of the cytosome to the nucleus, their elongated form suggestive of ameboid migration, and their prominent double nucleoli, the so-called amphinucleoli. One of these enlarges greatly during the growth period, the other much less. The larger of the nucleoli is regarded as being plasmatic; the smaller one, a true nuclear nucleolus.

The nucleus stains more lightly than the surrounding cytoplasm, and as growth progresses the nuclear chromatin, irregularly dispersed, tends to lose its staining properties to some degree and shows up as rather faint fluffy masses, similar to the condition described for *Polygyra* by Pennypacker (1930).

Full-grown oöcytes, as seen in their later position in the acinus

walls, have irregular polygonal forms because of the pressure of adjacent cells. They measure 70–75 microns in their longest diameter. The nucleus tends to have a rounded outline, but in some of the largest cells it may be quite irregular. This may indicate an approach to the breaking up in preparation for the maturation divisions. But I have never found any maturation spindles or cleavage stages while the female cells remained in the ovotestis of *Vallonia*, and a number of authors similarly record the absence of these (Byrnes, 1900; Larambergue, 1930; Pennypacker, 1930; Woldt, 1932). An exceptional case is that recorded by Lams (1910), who describes an intraovarian insemination in *Arion empiricorum*. He observed not only maturation divisions but segmenting eggs, morulae, and blastulae, in the interior of the gland.

*Maturation divisions.* — It seems evident that in *Vallonia*, as in the majority of the forms cited above, maturation divisions do not take place until after ovulation. The breaking up of the egg nucleus and the formation of the maturation spindle must be accompanied by a very rapid passage of the egg from the hermaphrodite gland into the outer genital passage. A number of authors record their failure to find ova in the hermaphrodite duct. In the present study maturation divisions have been observed only in eggs located in the outer passage, invested with albumen and shell, and ready to be laid. Such an egg is figured (text Fig. 5a) with what is interpreted as a polar view of the first maturation spindle, showing large asters and 32 chromosomes. The diploid number indicated by other counts is nearer 40. In this cell it may be that only part of the chromosomes have separated or that some are irregularly crowded together and difficult to distinguish.

Figure 5b shows an egg similarly situated in the uterus, with the second maturation division almost completed. The first polar body has migrated a short distance into the albumen. The second polar body still adheres to the surface of the egg, being held by remnants of spindle fibers. The egg chromosomes form a straggling group, not yet reorganized into the female pronucleus, and the sperm head, still small and compact in a little clear area near the periphery of the egg, has not yet begun its development into a male pronucleus. Indications are that the polar bodies have been given off in rapid succession, since the chromosomes of the first polar body are still clear and distinct. Counts of 17 and 18 have been made in several

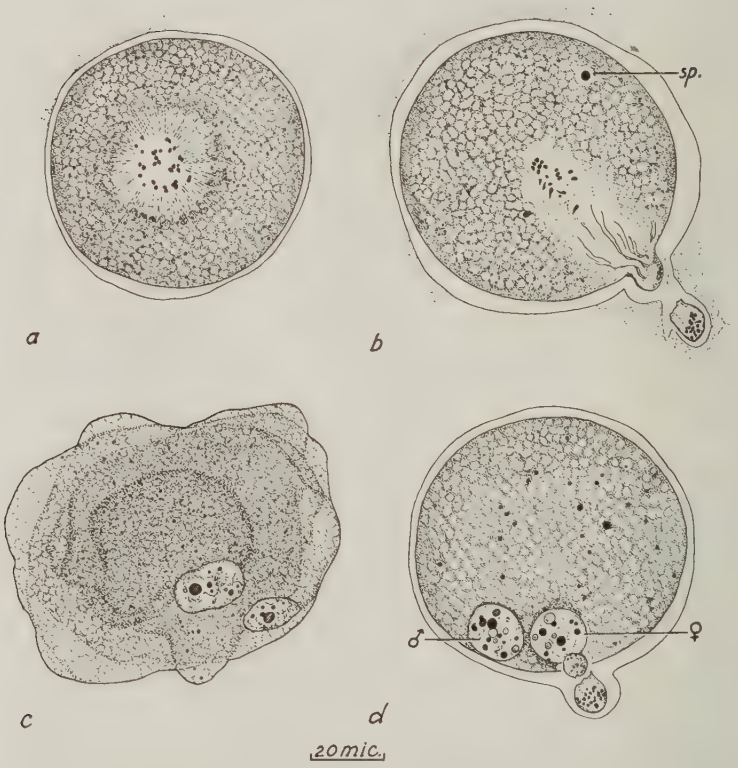


FIG. 5. Fertilized eggs found in outer genital passage: *a*, first maturation spindle, polar view of metaphasic chromosome plate; *b*, second maturation division — two polar bodies, egg chromosomes, and head of spermatozoon (*sp.*) shown — both *a* and *b* surrounded with albumen layer and shell; *c*, *d*, two fertilized eggs found in the same individual — *c* in the region of the albumen gland but lacking the albumen layer, *d* with albumen and shell — both showing male and female pronuclei. (All reconstructed from two or more adjacent sections)

instances. The chromosomes of the second polar body are smaller and less distinct. Few examples were obtained which were favorable for the counting of egg chromosomes. Those in Figure 5b number about 20.

### INSEMINATION

The intraovarian insemination noted in *Arion empiricorum* by Lams (1910) is exceptional among gastropods. In most studies authors have found insemination delayed until after oöcytes have left the acini. In *Vallonia* I have not observed actual penetration of spermatozoa, except possibly into early oöcytes (Pl. IV, Fig. 3), but it seems most reasonable to suppose that the definitive insemination occurs as the ova leave the acinus wall and traverse the medullary portion of the ovotestis on their way into the hermaphrodite duct. It is here, in the lumen of this medullary region, that male cells in all stages, including ripe spermatozoa, are abundant during the active season, and even a very rapid passage through this part of the gland would afford ample opportunity for insemination. Monospermy is evidently the rule. I have found no supernumerary spermatozoa in the uterine eggs or in the albumen surrounding them.

### FERTILIZATION

Good descriptions of fertilization processes in pulmonates have been contributed by Mark (1881) for *Limax campestris*, Garnault (1888–89) for *Helix aspersa* and *Arion empiricorum*, Byrnes (1900) and Linville (1900) for *Limax* species, Lams (1910) for *Arion empiricorum*, Crabb (1927) for *Lymnaea stagnalis appressa*, Larambergue (1930) for *Bullinus contortus*, and Perrot (1937) for *Helix pomatia*. There is a general similarity in these accounts. In the study of *Vallonia* it has not been possible to assemble a complete series of stages showing progressive steps in the development of the two pronuclei. The fact that eggs are deposited singly and at varying time intervals makes the obtaining of favorable stages a rather fortuitous matter. In only one instance have two eggs been found outside the hermaphrodite gland in the same individual. The first, enclosed in albumen and shell, occupies the lower passage (Fig. 5d). The second is farther back, in the region of the albumen gland but still lacking the albumen layer, and has an irregular form (Fig. 5c), which recalls a suggestion of Ikeda (1937) that ova make their way out of the hermaphrodite gland by means of ameboid movements. It has well-developed male and female pronuclei, like its predecessor, so that there is apparently no strict correlation between the progress

of maturation and fertilization and the level at which the ovum is found.

Such stages as have been obtained indicate that fertilization processes in *Vallonia* follow in a quite regular manner those of other pulmonates referred to above. There is only one spermatozoon in fertilized eggs; it appears first as a small, compact sperm head (Fig. 5b), but later transforms into a male pronucleus (Figs. 5c–d). The majority of the fertilized eggs in the uterine passage present in section an appearance like that of Figure 5d. They are invested with a thick albumen layer and shell, and are spherical, possibly because of suspension in the liquid medium, and also in part because of cortical changes accompanying fertilization. They measure 70–75 microns in diameter, which is the approximate size of the largest oöcytes while still in the hermaphrodite gland. This indicates that no growth takes place between the gland and the uterus. The cytoplasm is vesicular in appearance and contains small spherical yolk granules which stain black with iron hematoxylin.

The male and female pronuclei are typically in close juxtaposition at the animal pole. The male pronucleus in some preparations is slightly larger than the female and slightly elongated. The female pronucleus may be identified by the proximity of the two polar bodies. In both pronuclei the chromatin is in large, rounded, nucleoli-like masses, with smaller granules interspersed. This appearance must be quite characteristic, since it is noted repeatedly in the studies mentioned above.

In a few individuals cleavage has been found to occur while the egg is still in the oviduct. This may indicate an incipient tendency toward ovoviviparity. The eggs are retained in oviducts for varying periods, so that successive cleavages may take place. One egg fixed with the parent animal just as it was being laid shows indications of gastrulation. Others fixed soon after deposition are still in morula and blastula stages.

The evidence of typical chromosome reduction, the regular appearance of two polar bodies as well as two pronuclei, and the similarity of the stages studied to normal stages described for other pulmonates, together with the absence of the vas deferens and failure to observe copulation, all indicate that self-fertilization is the normal and usual process of reproduction in *Vallonia*.

DISCUSSION

One is impressed with the similarity of conditions in *Vallonia* to those of other Pulmonata, both in general and as regards many details. Specializations are noted, however, some of which at least seem to be due to restrictions of small size and others to habit and habitat. The large size of the eggs in proportion to that of the animal, their reduction in number, and protective features insuring juvenile survival are very striking. The eggs must be laid singly because of the small size of the animal, and egg laying is dependent on temperature and moisture. All land animals have to cope with problems of desiccation and extremes of temperature, and this is particularly true of a surface-living form such as *Vallonia*, which does not burrow to any extent, as does *Helix*, for example. These limiting factors are offset by the protracted breeding season (eggs have been collected out of doors from May to October) and by the readiness with which they are deposited whenever favorable conditions present themselves. Such conditions evidently induce a general activity of the germ gland, with the result that male and female cells in various stages of maturation are to be found in it at practically all times, although in an arrested condition during cold and dry periods. It is thus possible to bring some of them to maturity very quickly, as witness the readiness with which eggs are deposited in the laboratory by specimens brought in during the winter. Reproduction is evidently not complicated by alternating male and female phases or any such cyclical condition. Periods of drought and low temperature are to be regarded as enforced seasons of reproductive cessation rather than any functional break in the process.

The absence of male ducts, making self-fertilization obligatory, represents a further simplification both structurally and functionally and, as has been suggested in other studies, may have favored survival and propagation. I have found populations of *Vallonia* year after year in the same restricted area and at times very congested. This I attribute to the limited migratory powers of the animal and to artificially favorable moisture conditions around dwellings which make possible more continuous egg laying and a consequent piling up of populations.

Human agencies also multiply opportunities for accidental dispersal which would be advantageous to a slow-moving form, and

the power of self-fertilization would enable any isolated individual transported into a new region by such means to start a new colony.

## SUMMARY

This paper presents histological studies of the hermaphrodite gland of *Vallonia pulchella* Müll. as correlated with the life cycle and habits of the animal. The following features are brought out:

1. The ovotestis of the mature animal has the form of an acinous or digitate structure, with a number of cysts opening into a common lumen and joining the hermaphrodite duct, as is typical of many hermaphrodite mollusks. The efferent ducts have not been studied in detail, but the absence of the vas deferens was noted in sections, in agreement with the findings of previous authors. The absence of copulation is thus explained, and also the fact that fertile eggs are laid as freely by isolated individuals as by those in mass cultures.

2. The microscopic features of the gland differ according to the seasonal activity. Material fixed during the active summer season shows all stages of maturation; dormant winter specimens indicate an arrested condition of the gland, whereas spring and fall material exhibits intermediate conditions. Both male and female cells, however, are present during all seasons. There is no evidence of an alternation of male and female phases or of any cyclical condition other than the enforced cessation of reproductive activity during cold and dry periods.

3. The ovotestis develops from a compact cellular body, which was first distinguished at about the time of hatching and which is composed of indifferent germ cells. It later assumes the form of a single hollow vesicle, which subsequently takes on a lobate structure and becomes subdivided into the acini composing the adult gonad. Accompanying this development is a differentiation of male, female, and nutritive (or follicle) cells from the indifferent cells of the early gonad. They continue to arise from time to time in adult life, whenever conditions are favorable.

4. Male cells, which are first identified as primary spermatogonia, undergo their maturation and development in the lumen of the ovotestis. Female cells, first identified as oöcytes, develop in the walls of the acini, held in place by follicular membranes produced by follicle and nurse cells. The medullary portion of the gland is

the most active in the production of new elements. There is apparently a migration of small oöcytes formed in this region into the acini, where the remainder of their growth period is spent.

5. There are mature spermatozoa in the gland at an early stage, when the animal is about half grown; full-grown oöcytes do not occur until adult size is reached, at which time egg laying begins. There is evidence of some penetration of young oöcytes by spermatozoa, but such spermatozoa apparently disintegrate and do not result in fertilization.

6. Maturation divisions and effective insemination of the eggs do not occur until after they leave the acinus wall.

7. Usually only one fertilized egg is found in the uterus at any one time, since the small size of *Vallonia* prevents the laying of more. Monospermy occurs, which leads to the formation of a typical male pronucleus.

8. Two polar bodies are regularly given off, either shortly before or after the egg becomes invested with albumen and a calcareous shell, and a female pronucleus develops along with the male pronucleus in a typical manner. Cleavage may begin before the egg is laid.

9. Chromosomal counts made in spermatogonia, primary spermatocytes, secondary spermatocytes, first polocytes, and eggs give a haploid number of 18 to 20.

10. Histological evidence from this study, including the presence of mature sperm and eggs in the same acini at practically all times, the absence of the vas deferens, typical synapsis and chromosome reduction, the formation of two polar bodies and the development of two pronuclei, supports previous studies in establishing self-fertilization as the normal method of reproduction in *Vallonia pulchella.*

11. In general, reproductive conditions are effectively adjusted to the small size of the animal and to environmental factors, and are apparently favorable to its distribution and survival.

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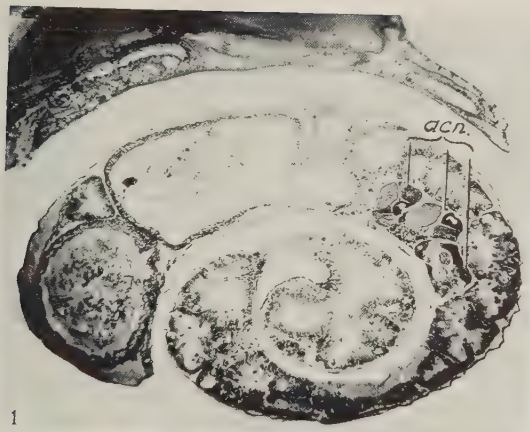
## EXPLANATION OF PLATE I

Abbreviations: *acn.*, acini; *al.*, albumen of egg; *al. gl.*, albumen gland; *fert. egg*, fertilized egg; *ut.*, uterus

Fig. 1. Sagittal section through mature *Vallonia pulchella*, showing the position of the hermaphrodite gland and the three groups of acini composing it

Fig. 2. Sagittal section of mature *Vallonia pulchella*, showing a fertilized egg in the uterus, ready to be laid

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## EXPLANATION OF PLATE II

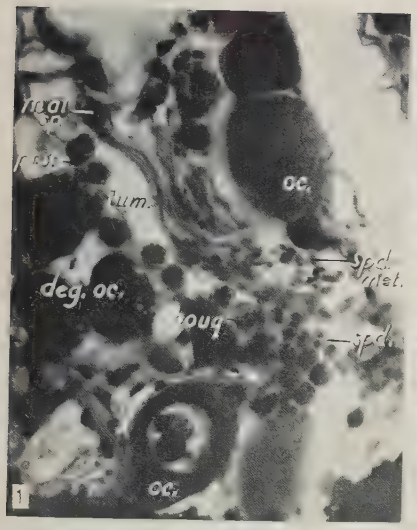
Conditions of the hermaphrodite gland at different seasons of the year

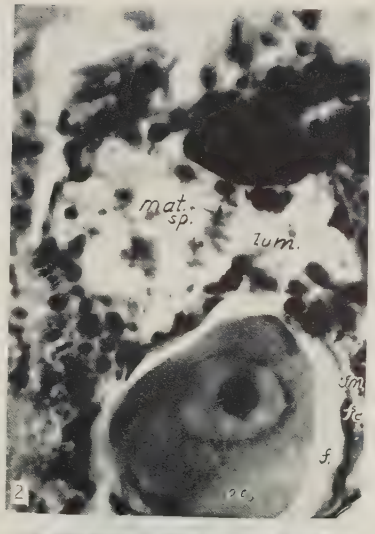
Abbreviations: *bouq.*, bouquet stage; *deg. oc.*, degenerating oöcytes; *f.*, follicle; *f. c.*, follicle cells; *f. m.*, follicular membrane; *lum.*, lumen; *mat. sp.*, mature spermatozoa; *oc.*, oöcytes; *pr. spc.*, primary spermatocytes; *spd.*, spermatids; *spd. met.*, spermatids in metamorphosis

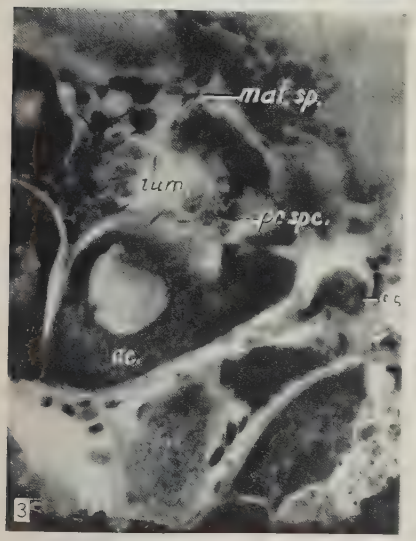
Fig. 1. Active medullary portion of gland in individual collected in July

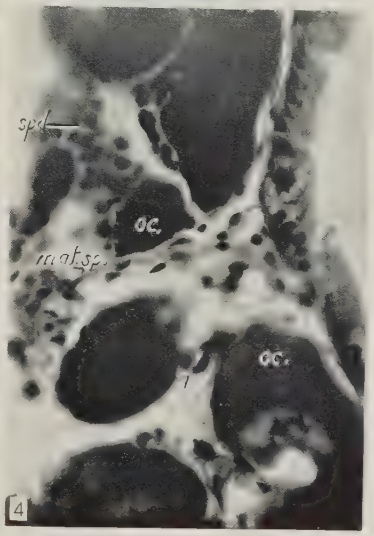
Fig. 2. Portion of gland in dormant individual collected during January

Figs. 3–4. Portions of the glands in individuals collected in April and October respectively


oc.
lum.
spd.
deg. oc.
spd.
oc.
1

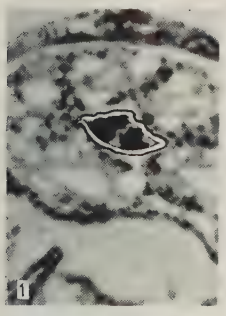

mat. sp.
lum.
2


mat. sp.
lum.
pc. spc.
oc.
3


spd.
oc.
mat. sp.
oc.
4

## EXPLANATION OF PLATE III

Series showing development of the hermaphrodite gland from the time of hatching up to and including adult size. Stages: Fig. 1, 0.6 mm.; Fig. 2, 0.9 mm.; Fig. 3, 1.0 mm.; Fig. 4, 1.3 mm.; Fig. 5, 1.5 mm.; Fig. 6, 1.8 mm.; Fig. 7, 2.0 mm. Measurements represent the widest shell diameter of the alcohol specimens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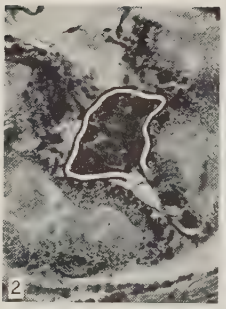
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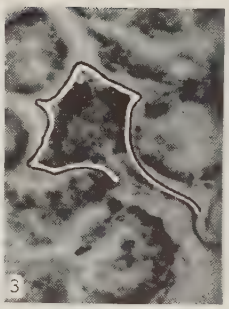
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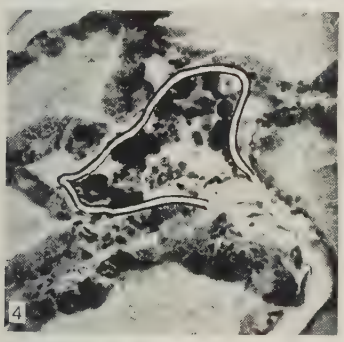
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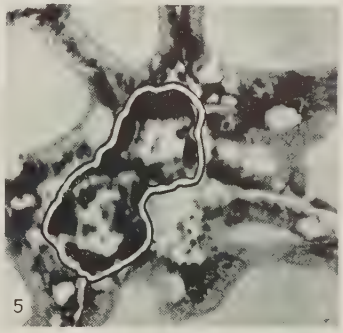
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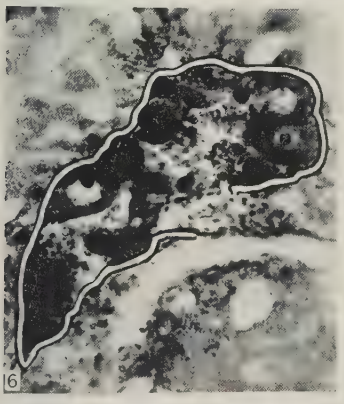
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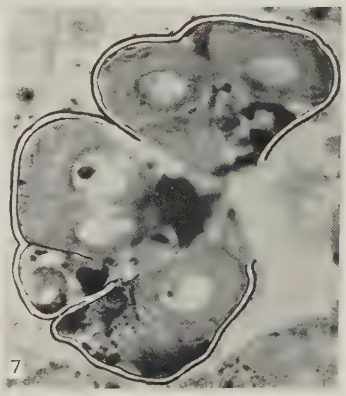
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## EXPLANATION OF PLATE IV

Oil-immersion studies of developmental stages

Abbreviations: *deg. oc.*, degenerating oöcytes; *f.*, follicle; *f. m.*, follicular membrane; *h. d.*, hermaphrodite duct; *lum.*, lumen; *mat. sp.*, mature spermatozoa; *oc.*, oöcytes; *prim. g. c.*, primordial germ cells; *pr. spc.*, primary spermatocytes; *pr. spg.*, primary spermatogonia; *sec. spc.*, secondary spermatocytes; *sec. spg.*, secondary spermatogonia; *spd.*, spermatids

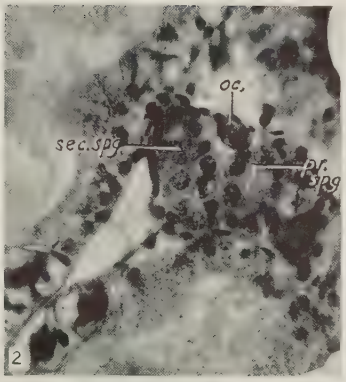
FIG. 1. Undifferentiated early gonad shortly after hatching

FIG. 2. Beginning of differentiation, as seen in the 0.9-mm. individual (early oöcytes at the periphery; early maturation stages of male cells in the interior)

FIG. 3. Further differentiation as seen in a half-grown (1.0-mm.) snail; ripe sperm present, some penetrating into the young oöcytes

FIG. 4. Portion of mature gonad, showing full-grown and smaller oöcytes and male cells in various stages of maturation

prim. g.c.
1


oc.
sec.spg.
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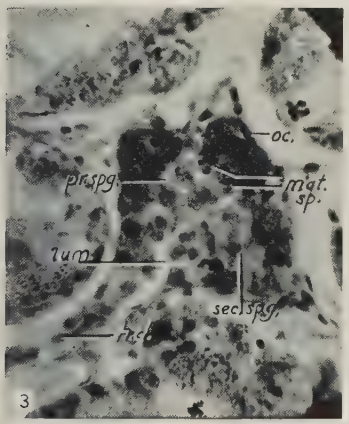
2


oc.
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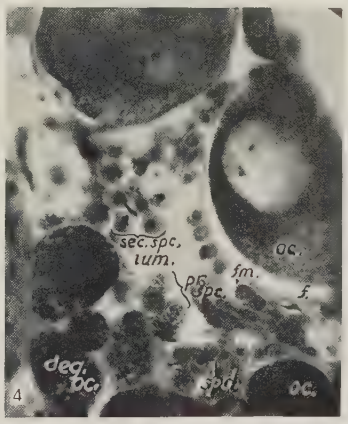
3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4

# A SERIES OF LATERAL ORGANS FOUND IN EMBRYOS OF THE SNAPPING TURTLE (*CHELYDRA SERPENTINA*)

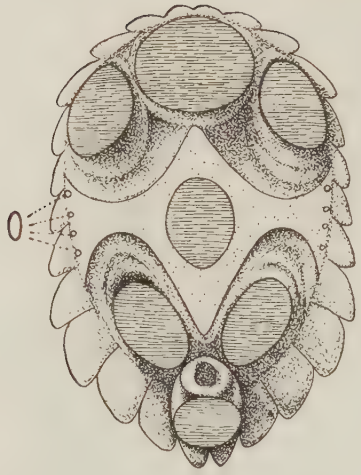
RAINER ZANGERL

PECULIAR organs serially arranged were noticed by the author along the lateral edges of the plastron in embryos of the snapping turtle *Chelydra serpentina*.[1] There are four in a row on either side of the body (Fig. 1). They are of such unusual histological structure that their morphological nature is difficult to determine. Each consists of an egg-shaped belly part, which lies within the subdermal mesenchyme, and a duct, which connects the belly portion with the outside of the embryo. Their shape varies slightly, but their chief construction appears identical in all specimens so far observed.

Histologically the belly of each organ is composed of two distinct parts: a cavity which represents the enlarged end region of the outleading duct and a comparatively heavy individual muscle, which encloses the whole end portion of the tube (Pl. I, Figs. 1–2). The entire organ is covered with a tough connective-tissue sheath. In addition, there is a layer of loose supporting tissue between the muscles and the end piece of the duct.

The tubular part of these organs is lined by stratified columnar epithelium which, at the distal end of the tube, goes over into the epidermis of the skin. The proximal end of the tube is blind and enlarged, and thus forms a terminal cavity. Some of the epithelial cells in this region (and also in the duct) seem to have freed themselves from their epithelial basis and appear within the lumen of the epithelial ingrowth. These cells are irregular in shape, but it is hard to decide whether they carry protoplasmic processes or whether the structures observed are due to a shrinkage of the cell bodies. True

[1] The writer is greatly indebted to Mr. H. Seton, of the Agassiz Museum at Harvard University, for a number of snapping-turtle embryos used for this investigation. They belonged to one litter, and their average size was about 20 millimeters carapace length.



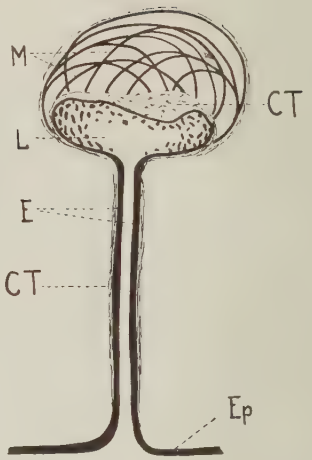




Fig. 1 Fig. 2

Fig. 1. Embryo of *Chelydra serpentina* (carapace length about 20 mm.) seen from the ventral side. The head, feet, and tail are cut off

Fig. 2. Diagrammatical drawing of an organ in cross section

Explanation of abbreviations: CT, connective-tissue sheath enclosing the organ and separating the end portion of the tube from the muscle; E, epithelium of the tube region; Ep, epidermis of the skin; L, lumen of the end piece of the duct; M, muscle bundles, with the distribution and the course of a few of them indicated; O, location of the organs in the embryo

cilia could not be found in the tube or in the blind end region. The question whether the cells lying within the lumen of this epidermal invagination have lost their normal epithelial connection because of maceration in the fixation fluid (the specimens were kept in formalin) can be answered in the negative with some certainty. There are no other places in the sections where a clear dislocation of cells due to maceration might have occurred. Furthermore, the epithelium lining the cavity and the duct appears in good contact; there are but very few spaces to be seen where cells might have dropped out (Pl. I, Fig. 1).

Around the enlarged end region of the epithelial tube is a layer of loose connective tissue, the thickness of which varies in different places. It may be just a thin sheath or, especially on the dorsal surface of the blind tube end, a thick cushion (Pl. I, Fig. 1). It

seems to be the place of origin and insertion of the relatively large muscle coat which forms the major portion of the belly part of the organ. The muscle cells are spindle-shaped and have a clear longitudinal striation. Not all the muscle cells run parallel, but the vast majority of them are transversally arranged. The course of the muscle-cell bundles is rather complicated. A few of them are drawn in Figure 2 to show their origins and insertions as well as their relationship to each other.

The organs are innervated by branches of the regionally corresponding spinal nerves which enter the belly portions at their anterior ends. Whether or not the nerves which innervate the organs carry sensory fibers besides motor ones has not been determined. In front of each organ the spinal nerve divides into two branches, one of which enters the organ; the other finds its way into the dermis.

A number of capillaries can be noticed throughout the muscle and connective-tissue region. Many white blood corpuscles occur, primarily along the peripheral edge of the muscle. They are ameboid in shape and have highly granulated nuclei. Similar cells are found in enormous quantities in the liver.

The unusual structure and location of these organs leave doubt as to their morphological and physiological nature. They might be glands, but the epithelium in the end region of the tube is not typically glandular and, furthermore, the presence of a heavy muscle belly around the end piece of a gland would be a unique feature. The structures might be sense organs of some kind. In view of their location, along the lateral edge of the body, one might think them to be organs of the lateral line system. There are no such organs known in Amniota, however. This interpretation becomes unsatisfactory also because of the association of muscles with these structures. A definite conclusion concerning the real nature of the organs can hardly be arrived at until new material for further study is available.

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PLATE I

FIG. 1

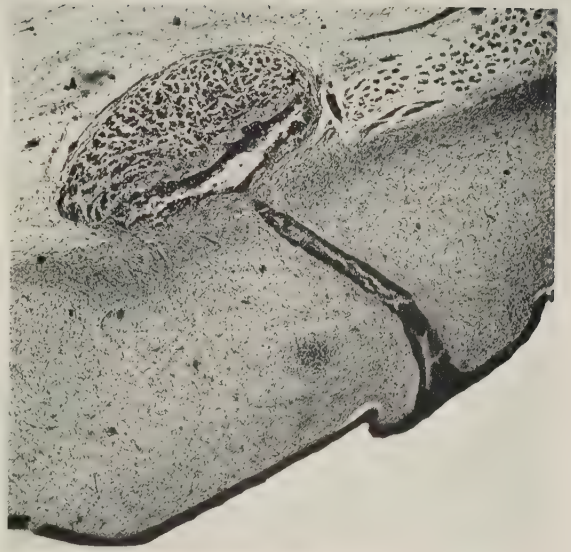

FIG. 2

Microphotographs of lateral organs in embryos of the snapping turtle: cross section (left); longitudinal section as seen in a parasagittal section through the embryo (right)